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The instrument is marked with this symbol where **high voltages** are present.

The instrument is marked with this symbol where **hot surfaces** are present.

The instrument is marked with this symbol where the user should refer to this *User's Guide* for instructions which may prevent damage to the instrument.

Warnings are given throughout this manual where care is required to avoid personal injury.

This manual is a companion to the *MassLynx NT User's Guide* supplied with the instrument.

All information contained in these manuals is believed to be correct at the time of publication. The publishers and their agents shall not be liable for errors contained herein nor for incidental or consequential damages in connection with the furnishing, performance or use of this material. All product specifications, as well as the information contained in this manual, are subject to change without notice.

Issue 2
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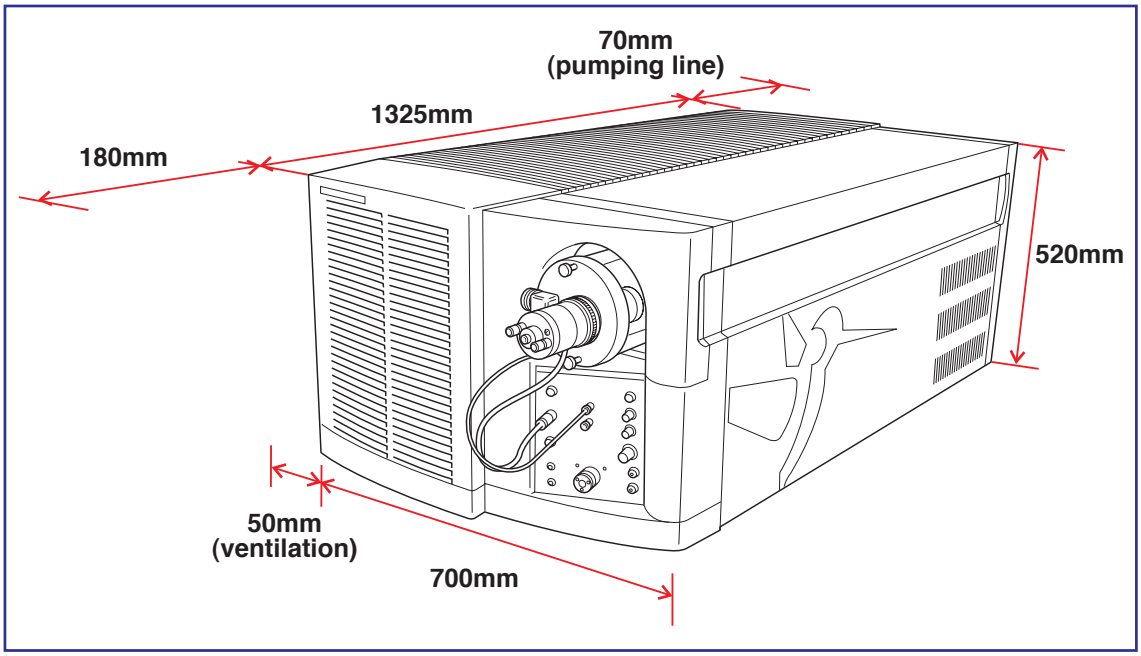
Hardware Specifications

Dimensions



Weights

Instrument:	150kg (330lb)
Data system (computer, monitor and printer):	60kg (130lb)
Rotary pump:	40kg (90lb)
Transformer (optional):	100kg (220lb)

Lifting and Carrying

Warning: Persons with a medical condition, for example a back injury, which prevents them from handling heavy loads should not attempt to lift the instrument.

Before lifting the instrument proceed as follows:

Vent and power down the instrument.

Disconnect the instrument from the power and water supplies.

Disconnect power and tubing connections to the rotary pump from the rear of the instrument.

Disconnect the API gas inlet and the exhaust lines from the rear of the instrument.

Disconnect all connections to LC equipment.

If the instrument is to be moved over a large distance or in a confined space it is recommended that any probes are removed from the API source.

The weight of the instrument is 150kg (330lb). Lifting equipment or suitably trained personnel will be required to lift or lower the instrument.

UK Health and Safety guidelines recommend that a minimum of six trained and suitable personnel are required to lift a unit of this weight. The instrument should be lifted from underneath the frame with one person at each corner of the instrument supporting the instrument in line with, or close to, the feet upon which the instrument stands. Two further people should support the instrument centrally.

Caution: Under no circumstances should the instrument be lifted by the front moulded cover, the probe or the source enclosure.

Before undertaking any lifting, lowering or moving of the instrument:

- Assess the risk of injury.
- Take action to eliminate the risk.
- Plan the operation.
- Use trained people.
- Refer to local or company guidelines before attempting to lift the instrument.

Micromass accept no responsibility for any injuries or damage sustained while lifting the instrument.

Power

Instrument:	230V (+10%, -14%), 13A
Data system:	100-120V or 200-240V, 13A
Power consumption:	3.0kW max.

Environment

Ambient temperature:	15-28°C (59-82°F)
Short term variance (1.5 hours)**:**	≤2°C (≤4°F)
Overall heat dissipation (excluding LC and optional water chiller)**:**	2.5kW maximum
Humidity:	Relative humidity ≤70%

Water Cooling

Heat dissipation into the water:	200W

Exhausts

Rotary Pump

The rotary pump must be vented to atmosphere (external to the laboratory) via a fume hood or industrial vent.

API Gas Exhaust

The API gas exhaust must be vented to atmosphere (external to the laboratory).

Caution: The API gas exhaust line must not be connected to the rotary pump exhaust line. In the event of an instrument failure, rotary pump exhaust could be admitted into the source chamber producing severe contamination.

Nitrogen

A supply of dry, oil-free nitrogen at 6-7 bar (90-100 psi) is required.

Caution: The lines supplying nitrogen to the instrument must be clean and dry. If plastic tubing is used it must be made of Teflon. The use of other types of plastic will lead to contamination of the instrument.

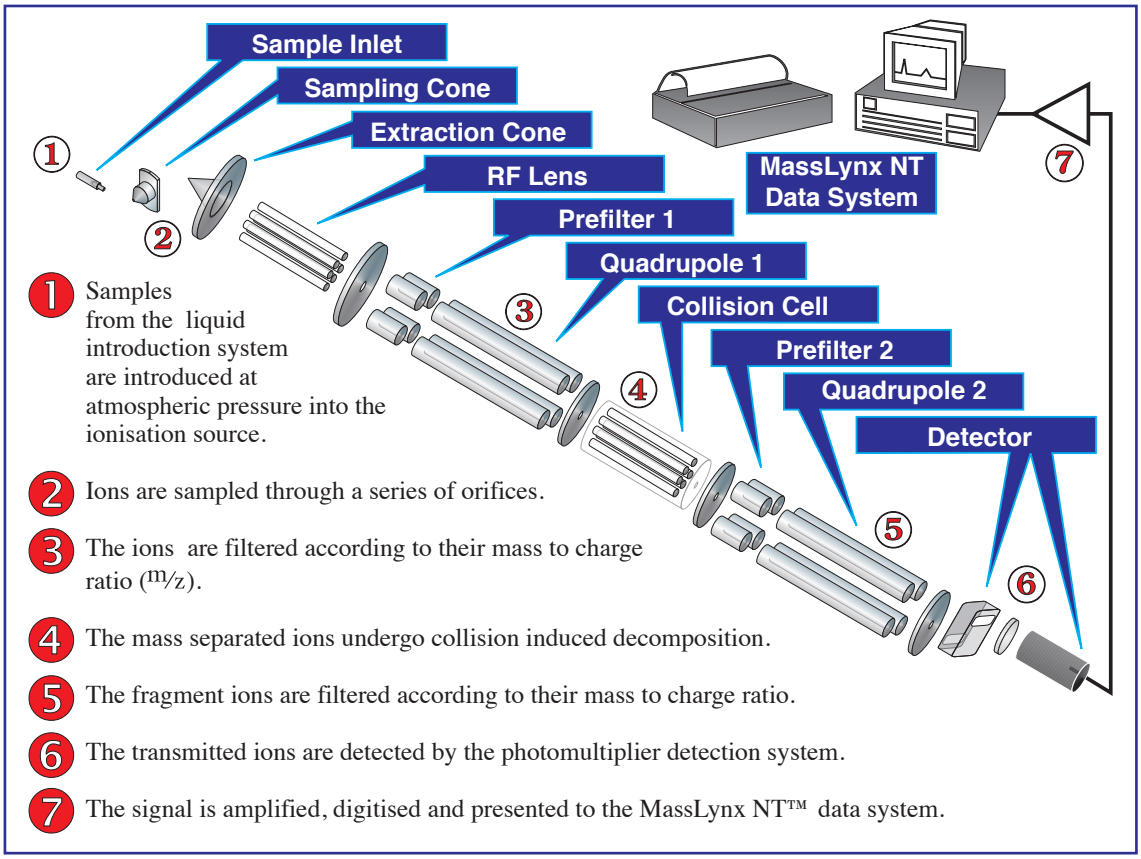
CID Gas

Argon is required as collision gas. The supplied gas should be dry (an in-line water trap is required), of high purity (99.9%) and at a pressure of approximately 350 mbar (5 psi).

Caution: Operating with the CID gas at a significantly higher pressure will result in a fault.

Instrument Description

Overview


Sample Inlet
Sampling Cone
Extraction Cone
RF Lens
Prefilter 1
Quadrupole 1
Collision Cell
Prefilter 2
Quadrupole 2
Detector
MassLynx NT
Data System
1 Samples from the liquid introduction system are introduced at atmospheric pressure into the ionisation source.
2 Ions are sampled through a series of orifices.
3 The ions are filtered according to their mass to charge ratio (m/z).
4 The mass separated ions undergo collision induced decomposition.
5 The fragment ions are filtered according to their mass to charge ratio.
6 The transmitted ions are detected by the photomultiplier detection system.
7 The signal is amplified, digitised and presented to the MassLynx NT™ data system.

The Micromass Quattro LC is a high performance benchtop triple quadrupole mass spectrometer designed for routine LC-MS-MS operation. Quattro LC may be coupled to:

- a HPLC system with or without an autosampler.
- an infusion pump.
- a syringe pump.

Ionisation takes place in the source at atmospheric pressure. These ions are sampled through a series of orifices into the first quadrupole where they are filtered according to their mass to charge ratio (m/z).

The mass separated ions then pass into the hexapole collision cell where they either undergo collision induced decomposition (CID) or pass unhindered to the second quadrupole. The fragment ions are then mass analysed by the second quadrupole. Finally the transmitted ions are detected by a conversion dynode, phosphor and photomultiplier detection system. The output signal is amplified, digitised and presented to the data system.

Vacuum System

Vacuum is achieved using a direct drive rotary pump, and two turbomolecular pumps.

The rotary pump, mounted on the floor external to the instrument, backs the turbomolecular pumps and also pumps the first vacuum stage of the source.

The turbomolecular pumps evacuate the analyser and ion transfer region. These pumps are both water cooled.

Vacuum measurement is by an active inverted magnetron (Penning) gauge for the analyser and a Pirani gauge for the gas cell. The Penning gauge acts as a vacuum switch, switching the instrument out of the OPERATE mode if the pressure is too high.

The speed of each turbomolecular pump is also monitored and the system is fully interlocked to provide adequate protection in the event of a fault in the vacuum system, a failure of the power supply or vacuum leaks.

Ionisation Techniques

Two atmospheric pressure ionisation techniques are available.

Atmospheric Pressure Chemical Ionisation

Atmospheric pressure chemical ionisation (APcI) generally produces protonated or deprotonated molecular ions from the sample via a proton transfer (positive ions) or proton abstraction (negative ions) mechanism. The sample is vaporised in a heated nebuliser before emerging into a plasma consisting of solvent ions and formed within the atmospheric source by a corona discharge. Proton transfer or abstraction then takes place between the solvent ions and the sample. Eluent flows up to 2 ml/min can be accommodated without splitting the flow.

Electrospray

Electrospray (ESI) ionisation takes place as a result of imparting a strong electrical charge to the eluent as it emerges from the nebuliser. An aerosol of charged droplets emerges from the nebuliser. These undergo a reduction in size by solvent evaporation until they have attained a sufficient charge density to allow sample ions to be ejected from the surface of the droplet (“ion evaporation”).

A characteristic of ESI spectra is that ions may be singly or multiply charged. Since the mass spectrometer filters ions according to their mass-to-charge ratio, compounds of high molecular weight can be determined if multiply charged ions are formed.

Eluent flows up to 1 ml/min can be accommodated although it is often preferable with electrospray ionisation to split the flow such that 100 to 200 μl/min of eluent enters the mass spectrometer.

Nanoflow Electrospray

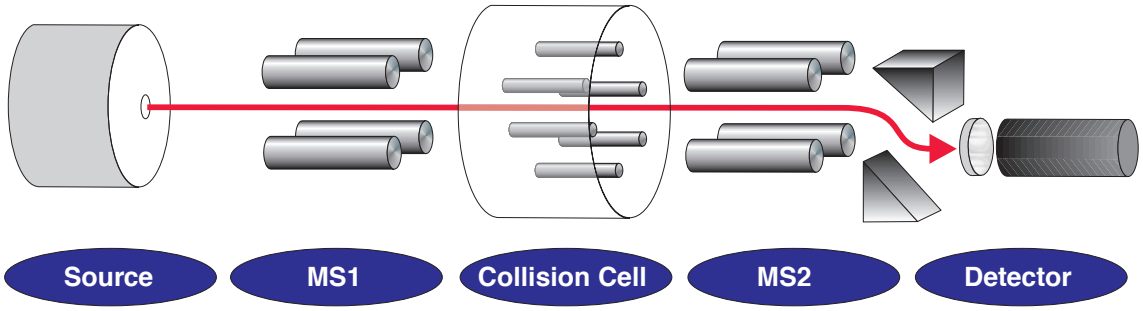
The optional nanoflow interface allows electrospray ionisation to be performed in the flow rate range 5 to 1000 nanolitres per minute.

For a given sample concentration, the ion currents observed in nanoflow are comparable to those seen in normal flow rate electrospray. Great sensitivity gains are therefore observed when similar scan parameters are used, due to the great reductions in sample consumption.

Sample Inlet

Sample is introduced from a suitable liquid pumping system along with the nebulising gas to either the APcI probe or the electrospray probe. For nanoflow electrospray, metal coated glass capillaries allow the lowest flow rates to be obtained while fused silica capillaries are used for flow injection analyses or for coupling to nano-HPLC.

MS Operating Modes



	MS1	Collision Cell	MS2
MS	Resolving	RF Only (Pass all masses)	
MS2	RF Only (Pass all masses)		Resolving

The MS1 mode, in which MS1 is used as the mass filter, is the most common and most sensitive method of performing MS analysis. This is directly analogous to using a single quadrupole mass spectrometer.

The MS2 mode of operation is used, with collision gas present, when switching rapidly between MS and MS-MS operation. It also provides a useful tool for instrument tuning and calibration prior to MS-MS analysis, and for fault diagnosis.

MS-MS Operating Modes

The basic features of the four common MS-MS scan functions are summarised below.

	MS1	Collision Cell	MS2
Daughter Ion Spectrum	Static (parent mass selection)	RF only (pass all masses)	Scanning
Parent Ion Spectrum	Scanning		Static (daughter mass selection)
Multiple Reaction Monitoring	Static (parent mass selection)		Static (daughter mass selection)
Constant Neutral Loss Spectrum	Scanning (synchronised with MS2)		Scanning (synchronised with MS1)

The Daughter Ion Spectrum

This is the most commonly used MS-MS scan mode. Typical applications are:

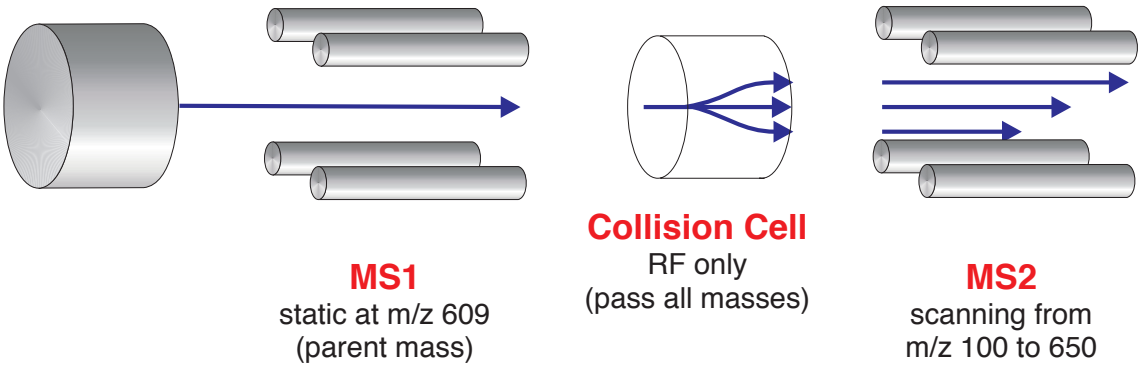
- Structural elucidation (for example peptide sequencing).
- Method development for MRM screening studies:

  Identification of daughter ions for use in MRM “transitions”.

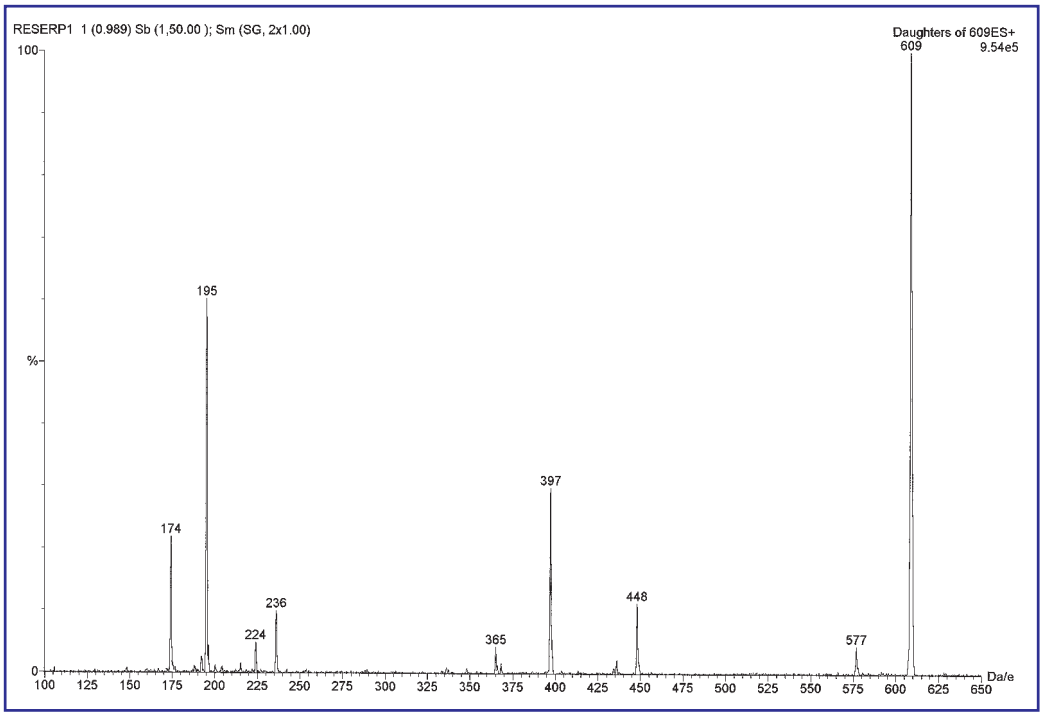
  Optimisation of CID tuning conditions to maximise the yield of a specific daughter ion to be used in MRM analysis.

Example:

Daughters of the specific parent at m/z 609 from reserpine in electrospray positive ion mode.



The result:



The Parent Ion Spectrum

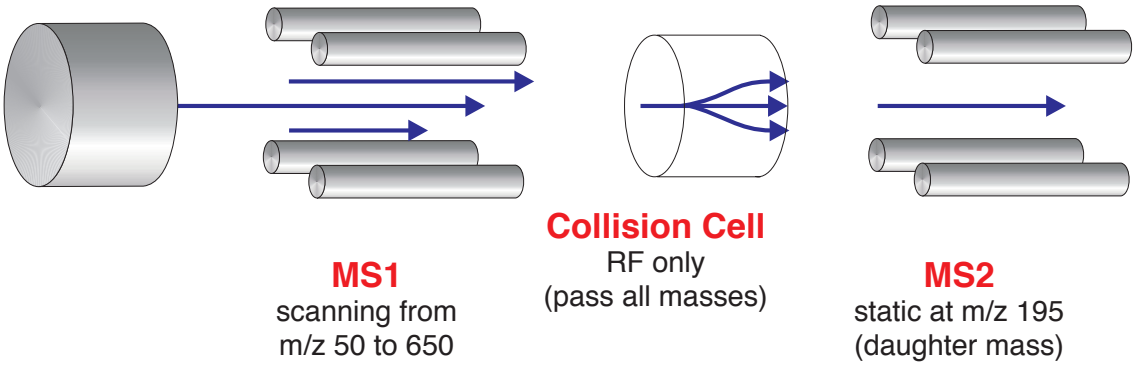
Typical application:

- Structural elucidation.

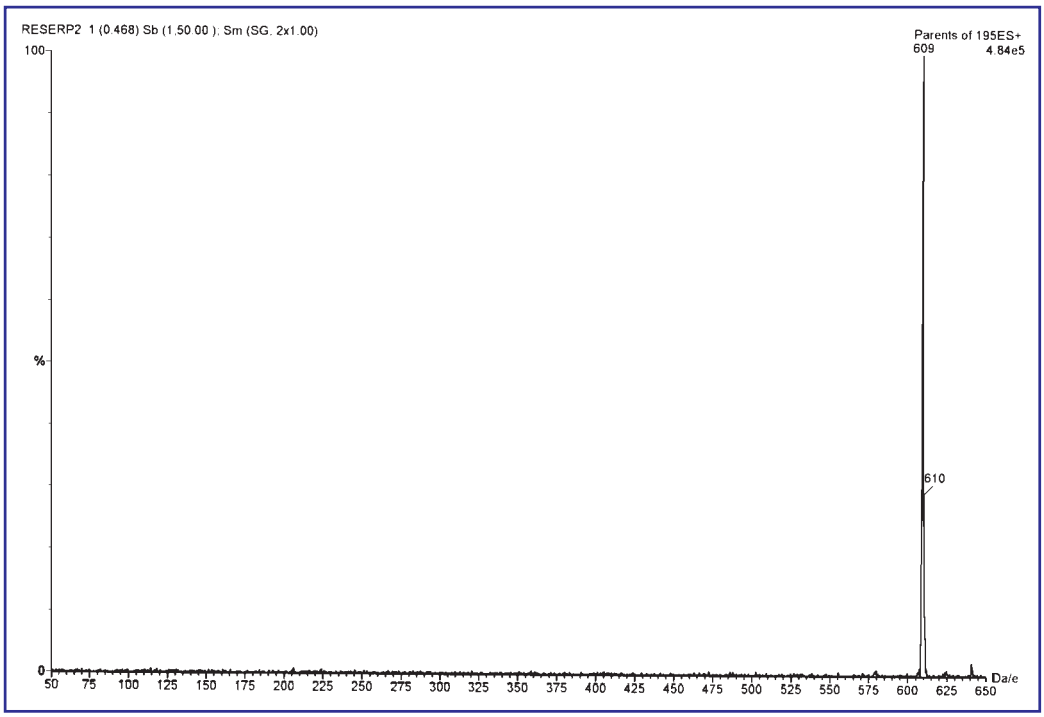
  Complementary or confirmatory information (for daughter scan data).

Example:

Parents of the specific daughter ion at m/z 195 from reserpine in electrospray positive ion mode.



The result:



MRM: Multiple Reaction Monitoring

This mode is the MS-MS equivalent of SIR (Selected Ion Recording). As both MS1 and MS2 are static, this allows greater "dwell time" on the ions of interest and therefore better sensitivity (~100×) compared to scanning MS-MS.

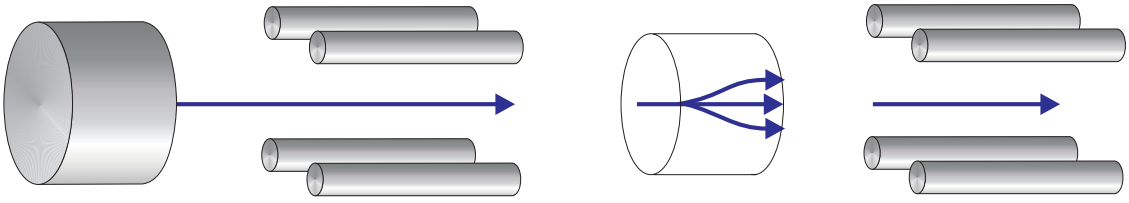
Typical application:

- Rapid screening of "dirty" samples for known analytes.

  Drug metabolite and pharmacokinetic studies
  Environmental, for example pesticide and herbicide analysis.
  Forensic or toxicology, for example screening for target drugs in sport.

Example:

Monitor the transition (specific fragmentation reaction) m/z 609 → 195 for reserpine in electrospray positive ion LC-MS-MS mode.



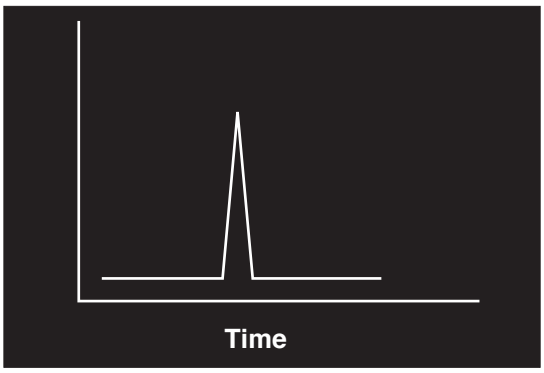
MS1
static at m/z 609
(parent mass)

Collision Cell
RF only
(pass all masses)

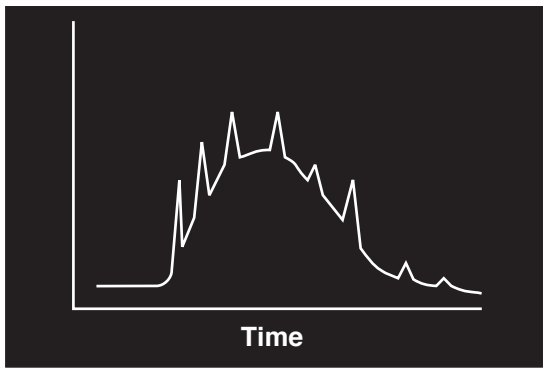
MS2
static at m/z 195
(daughter mass)

The result:

MRM does not produce a spectrum as only one transition is monitored. As in SIR, a chromatogram is produced.



LC-MRM
- High specificity
- Good signal / noise



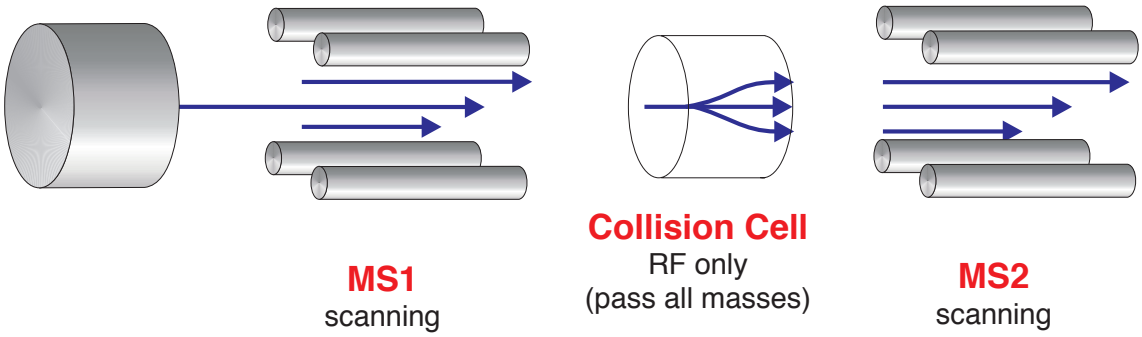
LC-MS
- Low specificity
- Poor signal / noise

The Constant Neutral Loss Spectrum

The loss of a specific neutral fragment or functional group from an unspecified parent or parents.

Typical applications:

- Screening mixtures, for example during neonatal screening, for a specific class of compound that is characterised by a common fragmentation pathway.



The scans of MS1 and MS2 are synchronised. When MS1 transmits a specific parent ion, MS2 "looks" to see if that parent loses a fragment of a certain mass. If it does it will register at the detector.

The result:

The "spectrum" will show the masses of all parents that actually lost a fragment of a certain mass.

Data System

The PC based data system, incorporating MassLynx NT™ software, controls the mass spectrometer detector and, if applicable, the HPLC system, autosampler, syringe pump, divert valve or injector valve.

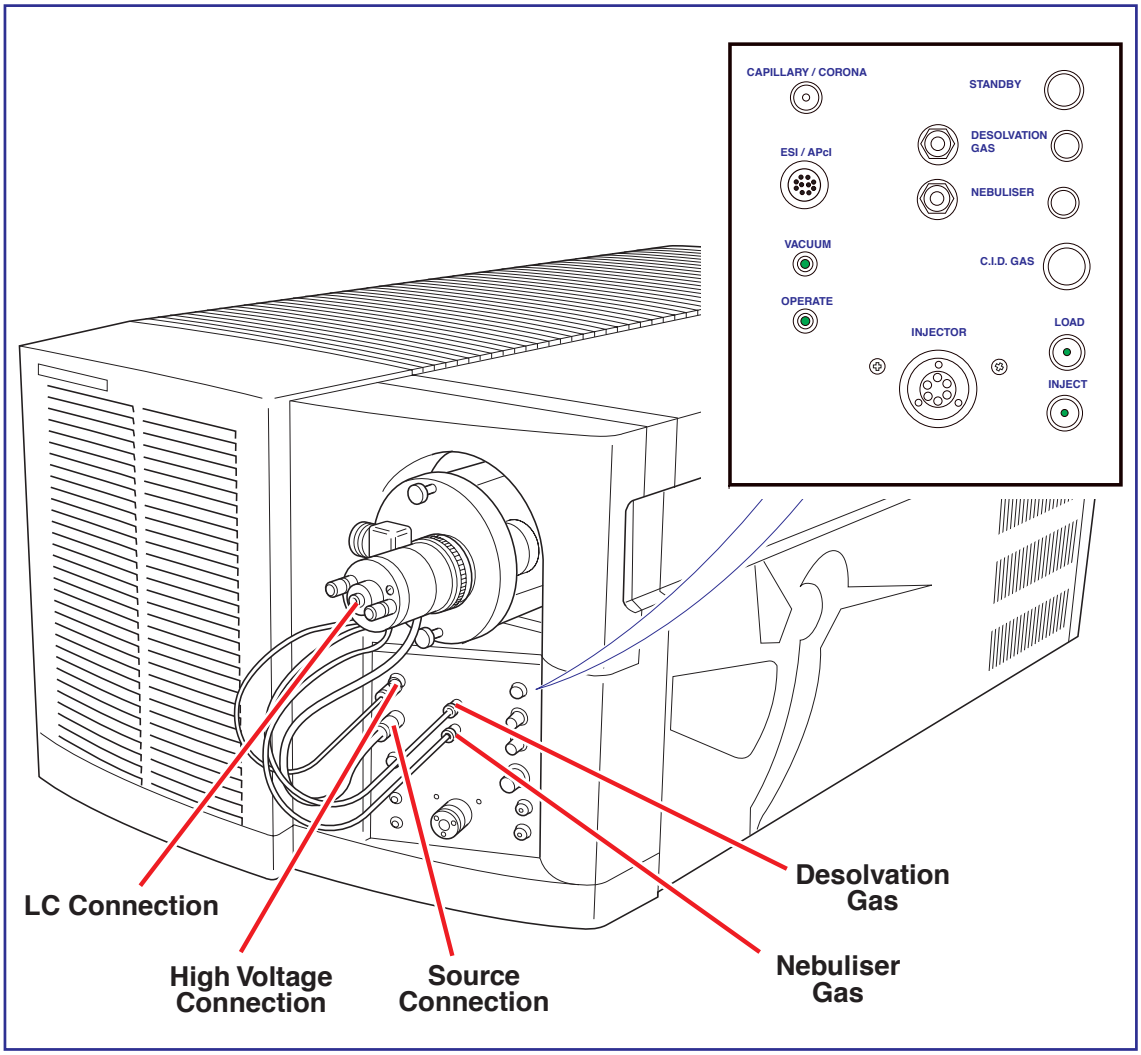
The PC uses the Microsoft Windows NT graphical environment with colour graphics and provides for full user interaction with either the keyboard or mouse.

MassLynx NT provides full control of the system including setting up and running selected HPLC systems, tuning, acquiring data and data processing.

Analog inputs can be read by the data system so that, where applicable, a trace from a conventional LC detector (for example UV or fluorescence) can be stored simultaneously with the acquired mass spectral data. A further option is the ability to acquire UV photodiode array detector data.

Comprehensive information detailing the operation of MassLynx NT is contained in the *MassLynx NT User's Guide*.

Front Panel Connections



Desolvation Gas and Probe Nebuliser Gas

The PTFE gas lines for the desolvation gas and probe nebuliser gas are connected to the front of the instrument using threaded metal fittings.

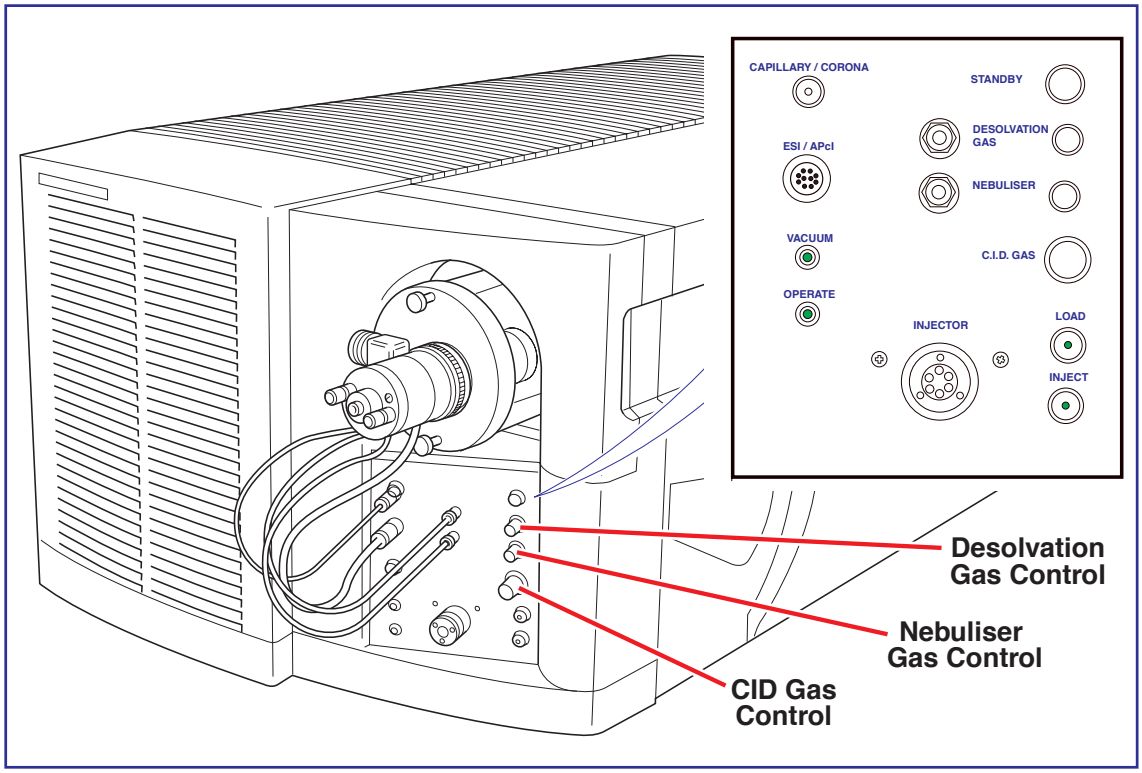
Capillary / Corona

The electrical connection for the ESI capillary or the APcI discharge pin is via the coaxial high voltage connector.

ESI / APcI

The electrical connection for the APcI probe or the ESI heater is via the multi-way connector. This is removed from the front panel by pulling on the metal sleeve of the plug. Both the electrospray and APcI heaters use this connector.

Front Panel Controls and Indicators



Status Display

The display on the front panel of the instrument consists of two 3-colour light emitting diodes (LEDs).

The display generated by the **Vacuum** LED is dependent on the vacuum status of the instrument. The **Operate** LED depends on both the vacuum status and whether the Operate mode has been selected from the Data System.

The status of the instrument is indicated as follows:

Vacuum LED

State	Vacuum LED		State	Vacuum LED
Vented	No indication		Vacuum OK	Steady green
Pumping	Steady amber		Pump fault	Flashing red

Operate LED

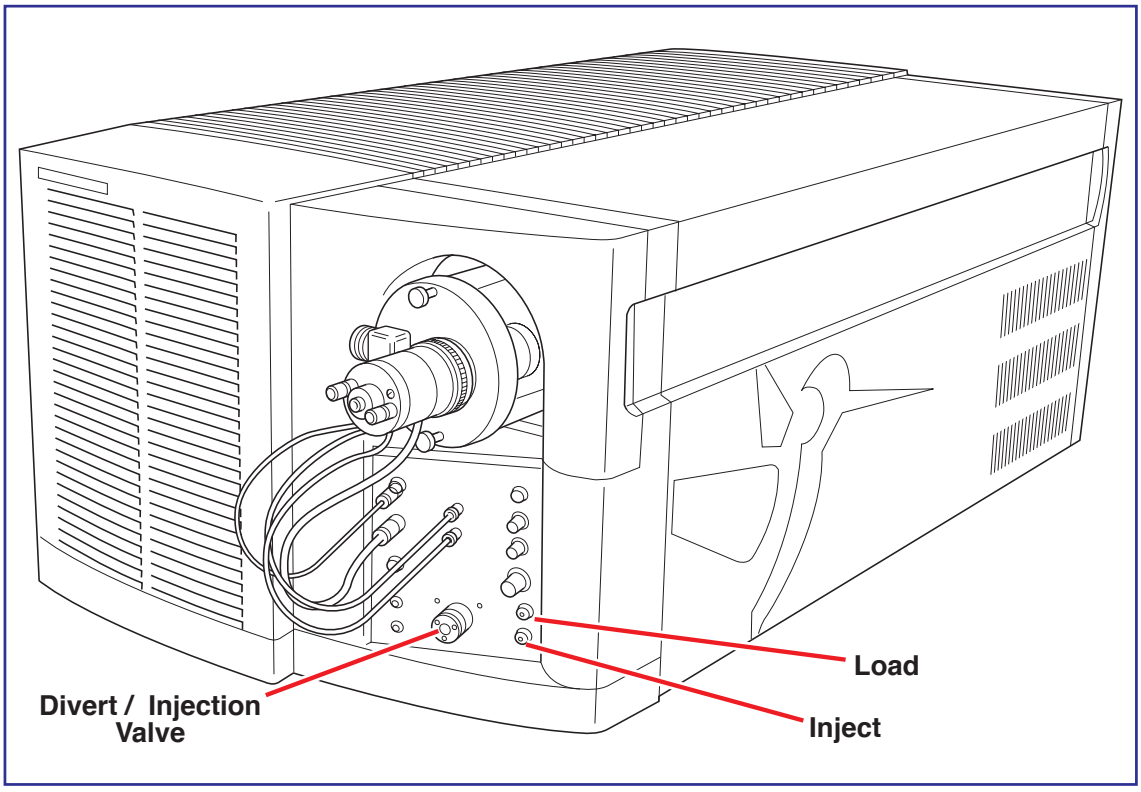
State	Operate LED		State	Operate LED
Standby	No indication		Transient pressure trip	Steady amber
Operate	Steady green		RF trip	Flashing red

Flow Control Valves

The **Desolvation Gas** and **Nebuliser** needle valves are five-turn valves. The **CID Gas** valve is a fifteen-turn valve. The flow increases as the valve is turned counterclockwise.

Caution: To prevent damage to the **CID Gas** valve, take care not to over-tighten when turning the supply off.

Divert / Injection Valve



The optional divert / injection valve may be used in several ways depending on the plumbing arrangement:

- As an injection valve, with the needle port and sample loop fitted.
- As a divert valve, to switch the flow of solvent during a LC run.
- As a switching valve to switch, for example, between a LC system and a syringe pump containing calibrant.

This valve is pneumatically operated, using the same nitrogen supply as the rest of the instrument.

Note that the valve is connected such that the nitrogen supply is always connected to the valve, irrespective of the flow to the source and probe.

Control of the valve is primarily from the data system. The two switches marked **Load** and **Inject** enable the user to override control of the valve when making loop injections at the instrument.

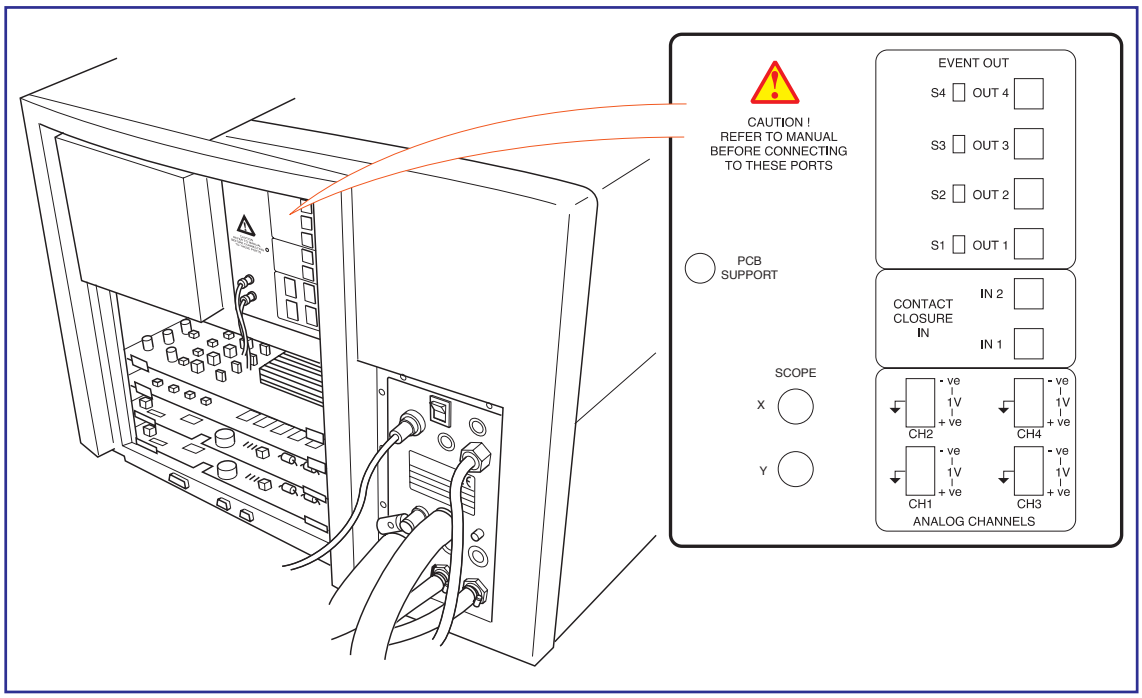
Rear Panel Connections

Event Out

Four outputs, **Out 1** to **Out 4**, are provided to allow various peripherals to be connected to the instrument. Switches **S1** to **S4** allow each output to be set to be either a contact closure (upper position) or a voltage output (lower position).

Out 1 and **Out 2**, when set to voltage output, each have an output of 5 volts. The voltage output of both **Out 3** and **Out 4** is 24 volts.

During a sample run **Out 1** closes between acquisitions, and is used typically to enable an external device to inject the next sample. The three remaining outputs are reserved for future developments.



Contact Closure In

In 1 and **In 2** inputs are provided to allow external device to start sample acquisition once the device has performed its function (typically sample injection).

Analog Channels

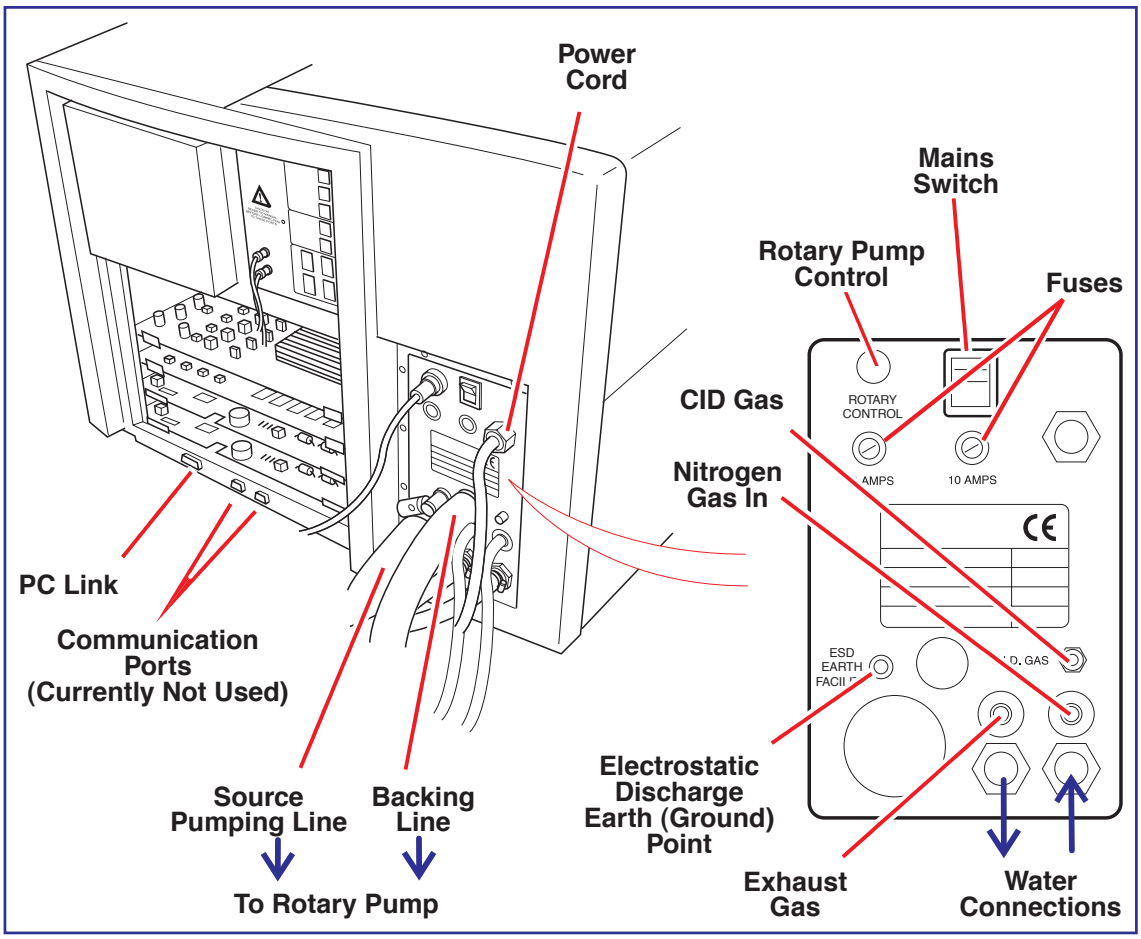
Four analog channel inputs are available, for acquiring simultaneous data such as a UV detector output. The input differential voltage must not exceed one volt, though full scale automatically adjusts from 1mV to 1V.

Scope

The **X** and **Y Scope** outputs are provided as a diagnostic tool for engineer's use, enabling peaks to be displayed on an oscilloscope. Vertical gain and display response are set via software.

The **X** output is a 0 to 5 volts ramp, 1kΩ output impedance.

The **Y** output is 0 to 10 volts full scale, 100Ω impedance.



Water

Water is used to cool the turbomolecular pumps.

Nitrogen Gas In

The nitrogen supply (100 psi, 7 bar) should be connected to the **Nitrogen Gas In** push-in connector using 6mm PTFE tubing. If necessary this tubing can be connected to ¼ inch tubing using standard ¼ inch fittings.

Caution: Use only PTFE tubing or clean metal tubing to connect between the nitrogen supply and the instrument. The use of other types of plastic tubing will result in chemical contamination of the source.

Exhausts

The exhaust from the rotary pump should be vented to atmosphere outside the laboratory.

The gas exhaust, which also contains solvent vapours, should be vented via a separate fume hood, industrial vent or cold trap.

The gas exhaust should be connected using 10mm plastic tubing connected to the push-in fitting.

Caution: Do not connect these two exhaust lines together as, in the event of an instrument failure, rotary pump exhaust could be admitted into the source chamber producing severe contamination.

CID Gas

Argon is required as collision gas. See *Hardware Specifications* for details.

Power Cord

The mains power cord should be wired to a suitable mains outlet using a standard plug. For plugs with an integral fuse, the fuse should be rated at 13 amps.

Mains Switch

The mains switch switches mains power to the instrument.

Fuses

Refer to *Maintenance and Fault Finding* for details of rear panel fuses, and all other instrument fuses.

Rotary Control

Mains power to the rotary pump is controlled by the data system using this socket.

ESD Earth Facility

A suitable wrist band should be connected to this point when handling sensitive electronic components, to prevent damage by electrostatic discharge.

Com1 and Com2

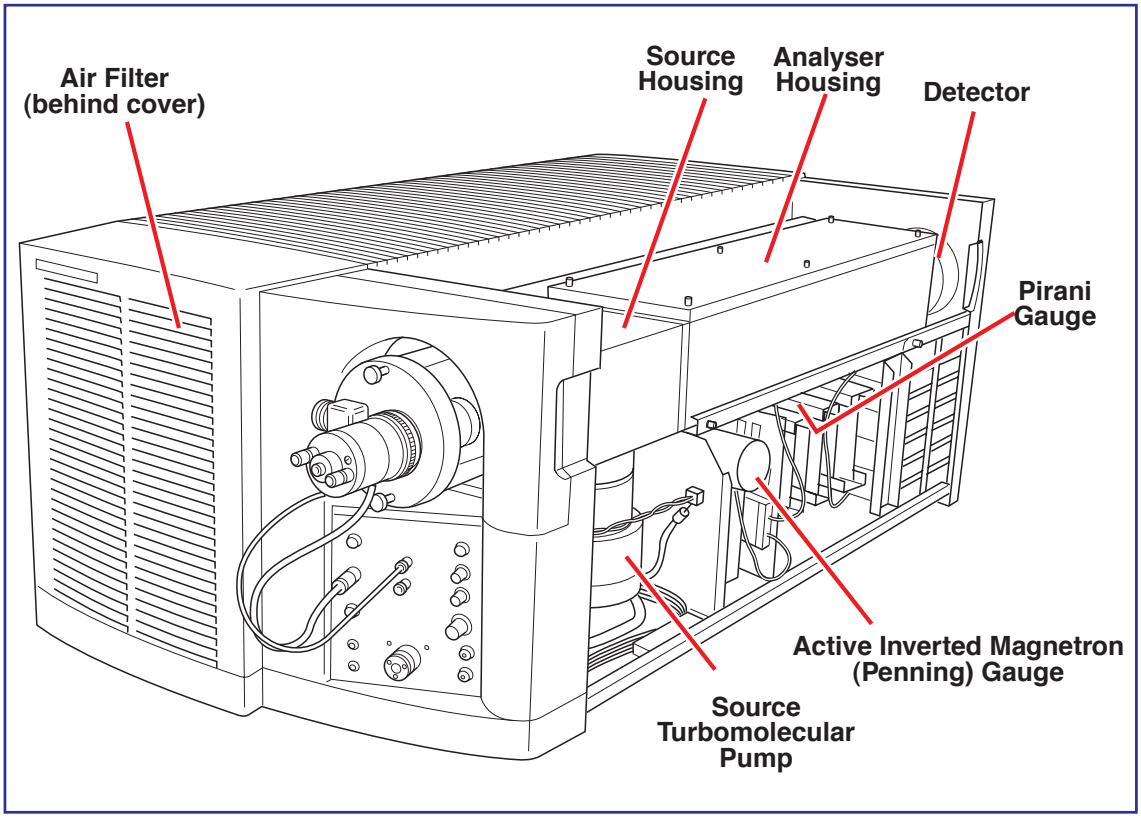
The two connections marked **Com1** and **Com2** are for communication with external devices, and are currently not used.

PC Link

The 15-way connector marked **PC Link** connects the instrument to the data system via the supplied link cable.

Internal Layout

Mechanical Components



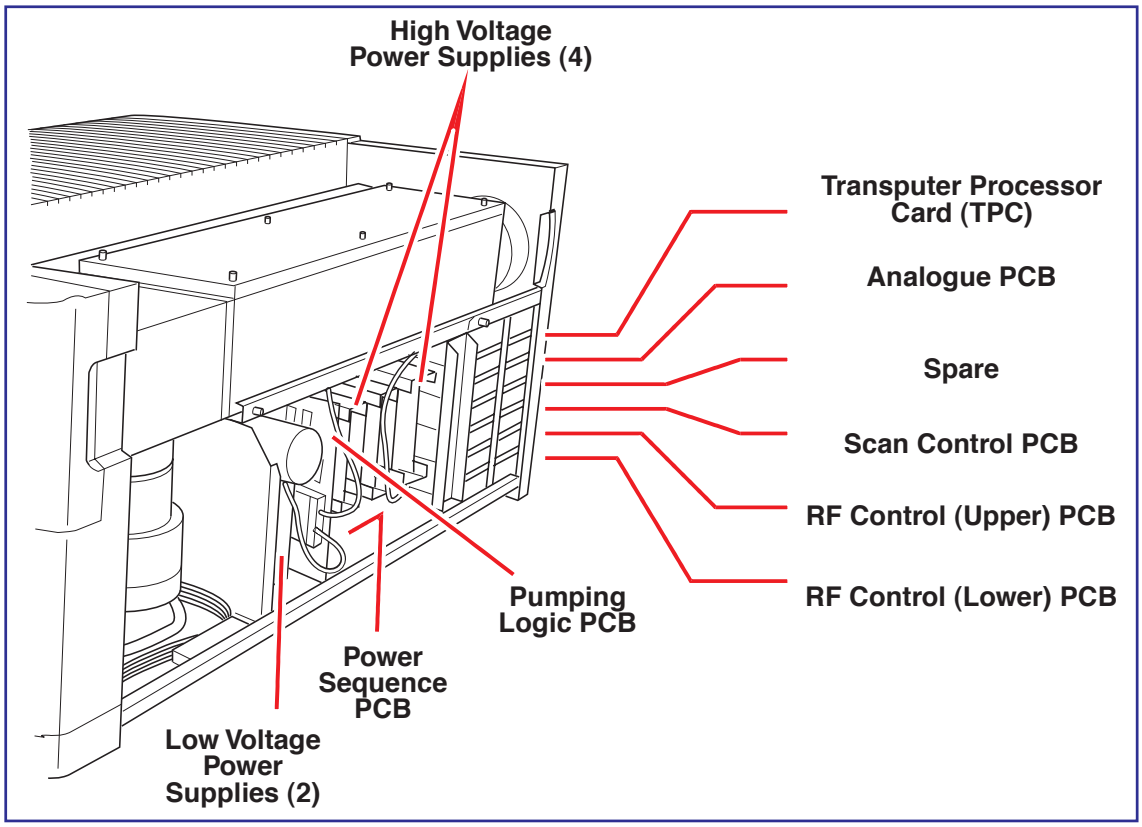
The main internal mechanical components of the instrument are:

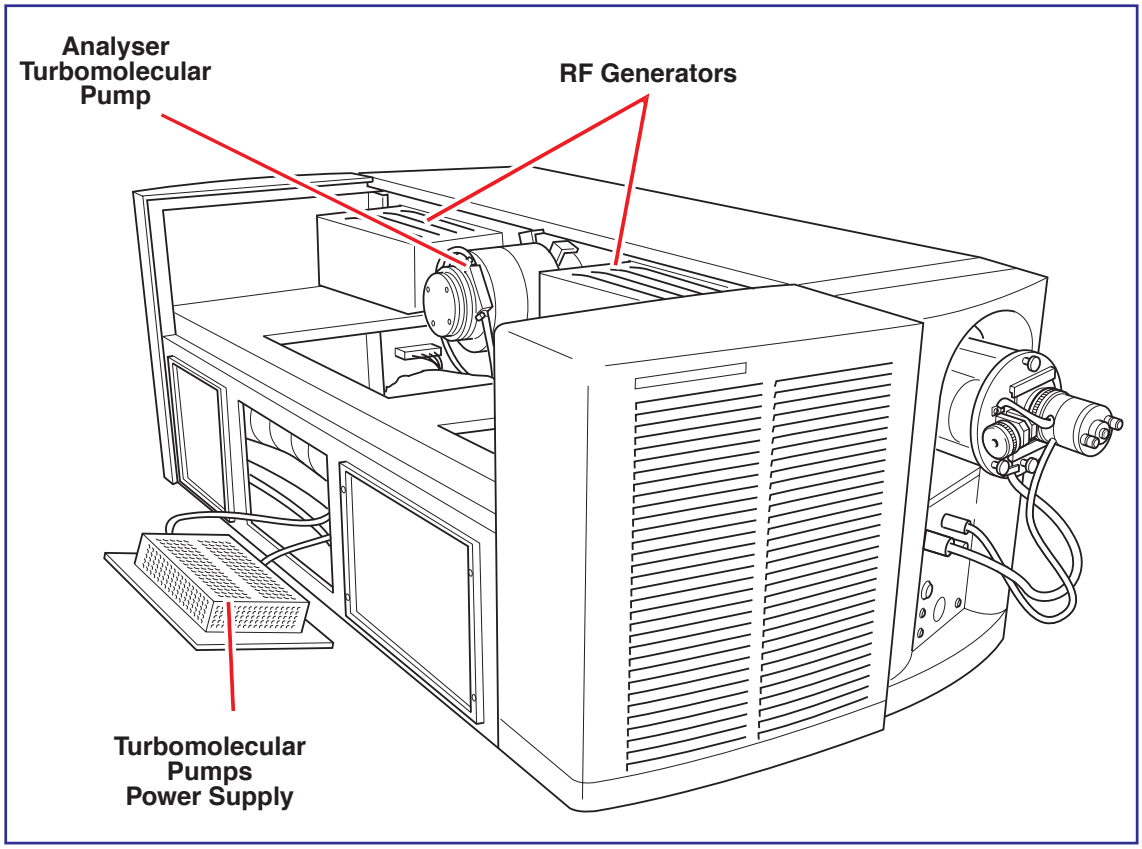
- The source housing, inside which is the hexapole transfer lens.

  The hexapole transfer lens is sometimes referred to as the "RF lens".

- The analyser housing, containing the two quadrupoles and the gas cell.
- The detector, attached to the rear of the analyser housing.
- Two 250 litre/second turbomolecular pumps, one pumping each of the above housings.
- The active inverted magnetron (Penning) gauge and the Pirani gauge, both clamped to the underside of the analyser housing.
- The air filter, held in the louvered cover at the left side of the front of the instrument.

Electronics





The main electronics modules of the system are:

- Two low voltage power supplies.
- Four high voltage power supplies, plugged into the backplane below the analyser housing.

  These supply the detector system and the high voltages for the source and electrospray probe.

- Two RF generators, bolted to the side of the analyser housing.
- Pumping Logic PCB.

  This controls the turbomolecular pumps, the pumping sequence, the gas valves and the solenoids. It also controls the phosphor and dynode voltages.

- Power Sequence PCB.

  This PCB examines the vacuum, operate and interlock signals in order to control the switching of various supplies. Also on this PCB is a module delivering the photomultiplier voltage.

- Transputer Processor Card (TPC).

  This contains the transputer array and controls data acquisition and control functions, as well as interfacing to the PC.

- Scan Control PCB

  This PCB produces control signals for mass, resolution, function energy, collision energy and pre-filter energy.

- Analog PCB

  This PCB controls the source heater and focusing voltages.

- RF Generator Control (Upper) PCB

  This controls the RF and DC voltages applied to the first quadrupole. It also supplies the collision cell voltages.

- RF Generator Control (Lower) PCB

  This controls the RF and DC voltages applied to the second quadrupole.

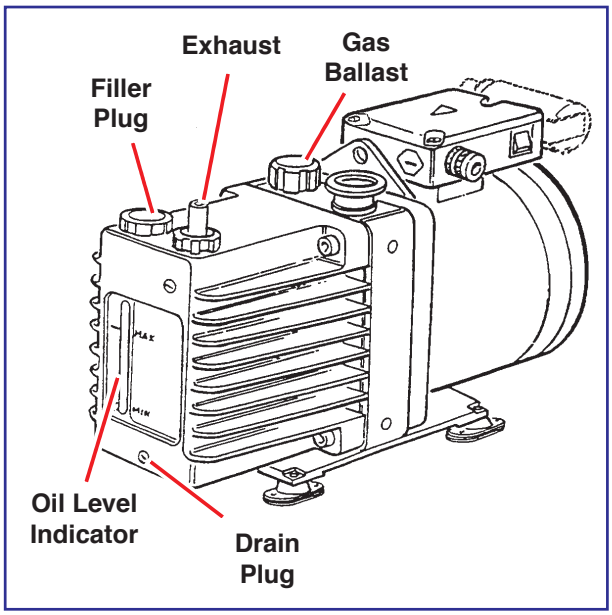
Routine Procedures

Start Up Following a Complete Shutdown

Preparation

If the instrument has been unused for a lengthy period of time, proceed as follows:

Check the level of oil in the rotary pump sight glass. Refill or replenish as necessary as described in the pump manufacturer's literature.



Connect a supply of dry, high purity nitrogen to the connector on the service panel at the rear of the instrument. Adjust the outlet pressure to 7 bar (100 psi).

Connect a supply of argon to the **CID Gas** connector on the service panel at the rear of the instrument. Adjust the outlet pressure to approximately 350 mbar (5 psi).

Connect the water supply to the connections at the rear of the instrument.

Check that the rotary pump control box is connected to **Rotary Control** at the rear of the instrument, and to the rotary pump.

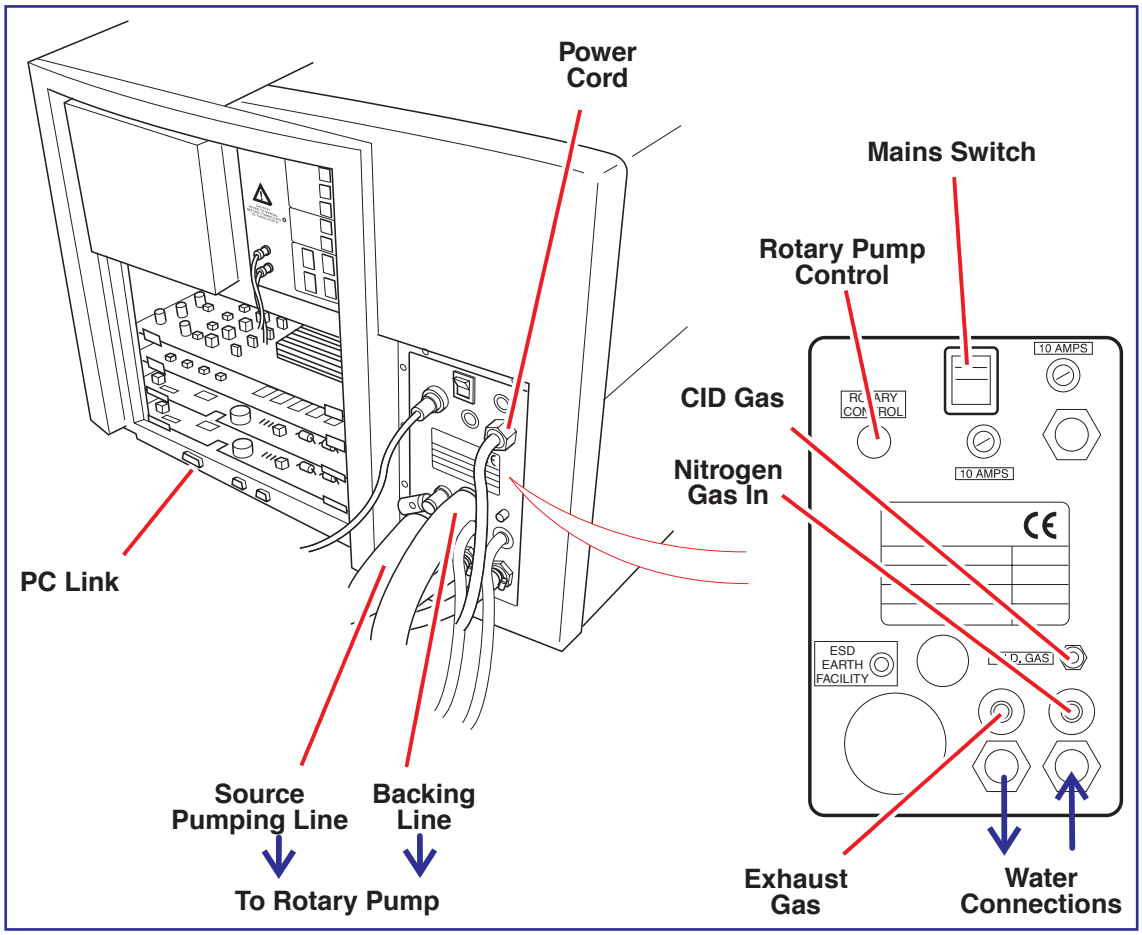
Check that the instrument, rotary pump control box, data system and other peripheral devices (LC equipment, printer etc.) are connected to suitable mains supplies.

Check that the data system is connected to the mass spectrometer via the **PC Link** cable.

Check that the rotary pump exhaust is connected to a suitable vent.

Check that the exhaust gas from the instrument is connected to a suitable vent. This must not be the same vent as the rotary pump exhaust.

Caution: Do not connect the two exhaust lines together. In the event of an instrument failure, rotary pump exhaust could be admitted into the source chamber, producing severe contamination.



Switch on the mains to the mass spectrometer using the switch situated on the service panel at the rear of the instrument.

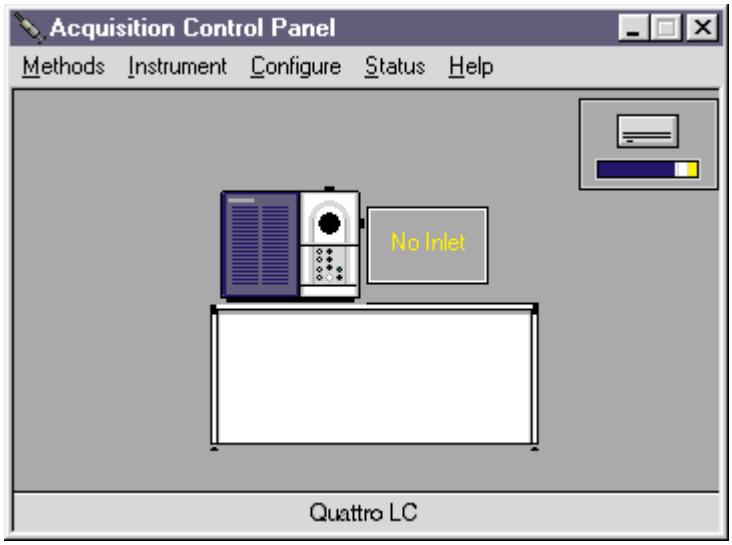
Switch on the data system.

As supplied Windows NT is automatically activated following the start-up sequence whenever the data system is switched on.

Windows NT and MassLynx NT can be configured to prevent unauthorised access. Consult the system administrator for any passwords that may be requested.

When the data system has booted up, double-click on the MassLynx icon in the Windows desktop.

Select **Run** and **Control Panel**, or press , to bring up the acquisition control panel.



A picture of the instrument is displayed.

To display the tune page either:

Double click on the mass spectrometer section of the instrument picture.

or:

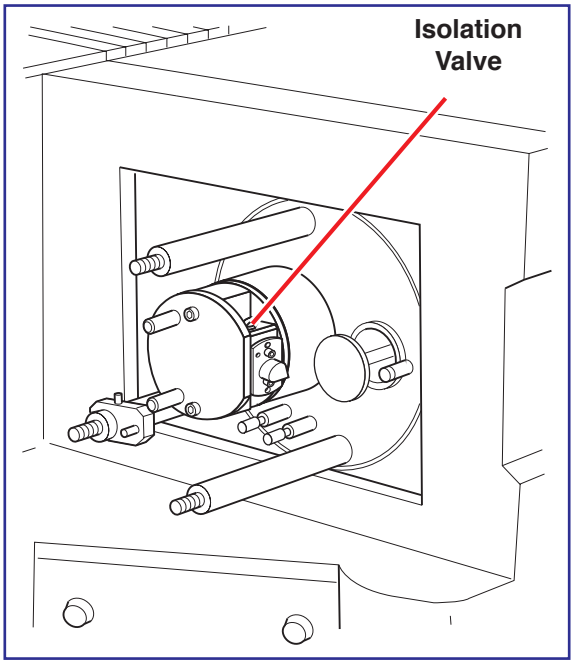
Select **Instrument**, then **Tune Mass Spectrometer** from the acquisition control panel.

Alternatively, by simply clicking on *, the tune page can be launched directly from the MassLynx top-level window.*

Pumping

Caution: To minimise wear to the lubricated components of the rotary pump, the manufacturers recommend that the pump is not started when the oil temperature is below 12°C.

Pump down time may be decreased by closing the isolation valve of the source during pump down.



Select **Other** from the menu bar at the top of the tune page.

Click on **Pump**.

The rotary pump and the turbomolecular pumps start simultaneously.

The **Vacuum** *LED on the front of the instrument shows amber as the system pumps down.*

When the system has reached operating vacuum the LED changes to a steady green, indicating that the instrument is ready for use.

If the rotary pump oil has been changed or replenished, open the gas ballast valve on the rotary pump. See the pump manufacturer's literature for details.

Rotary pumps are normally noticeably louder when running under gas ballast.

If opened, close the gas ballast valve when the rotary pump has run under gas ballast for 30 minutes.

Using the Instrument

Quattro LC is now almost ready to use. To complete the start up procedure and prepare for running samples, follow the instructions in *Start Up Following Overnight Shutdown* in the following pages.

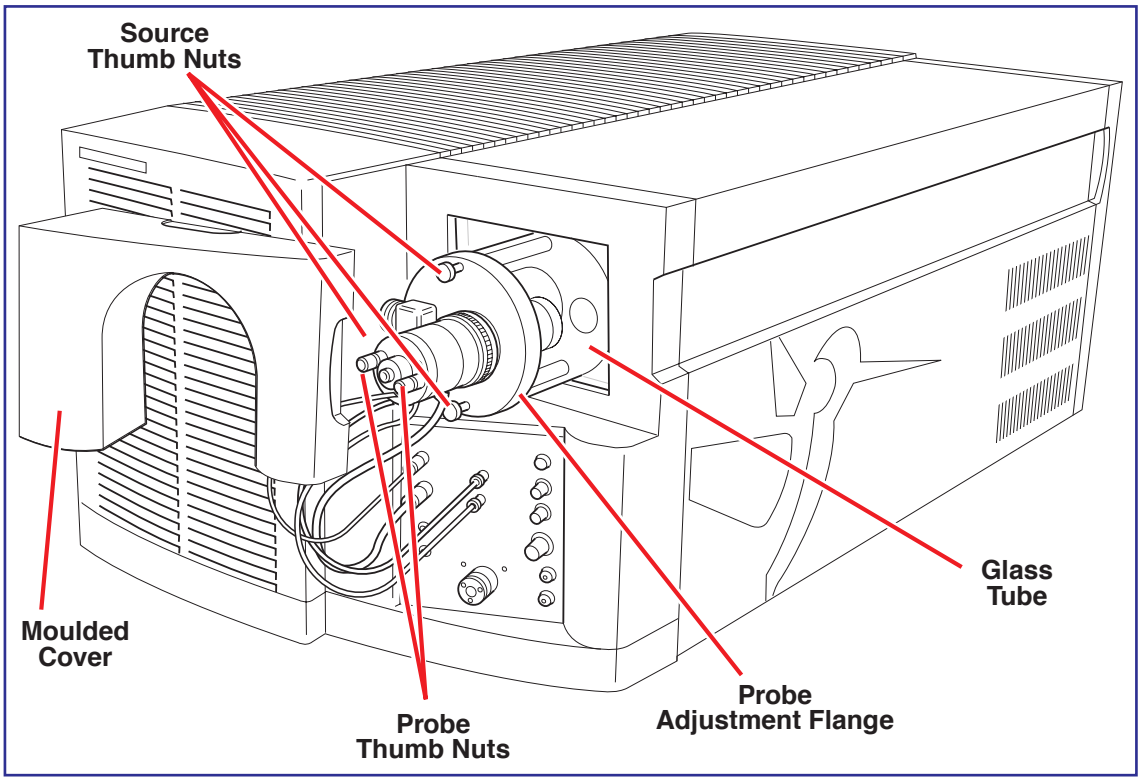
Start Up Following Overnight Shutdown

The instrument will have been left in standby mode under vacuum.

It is recommended that the data system is left on overnight. However, if the data system has been switched off, switch it on as described in the preceding section.

The display on the front of the instrument displays a steady green **Vacuum** *LED indicating that the instrument is ready for use.*

Preparation for Electrospray Operation



If the corona discharge pin is fitted, proceed as follows:

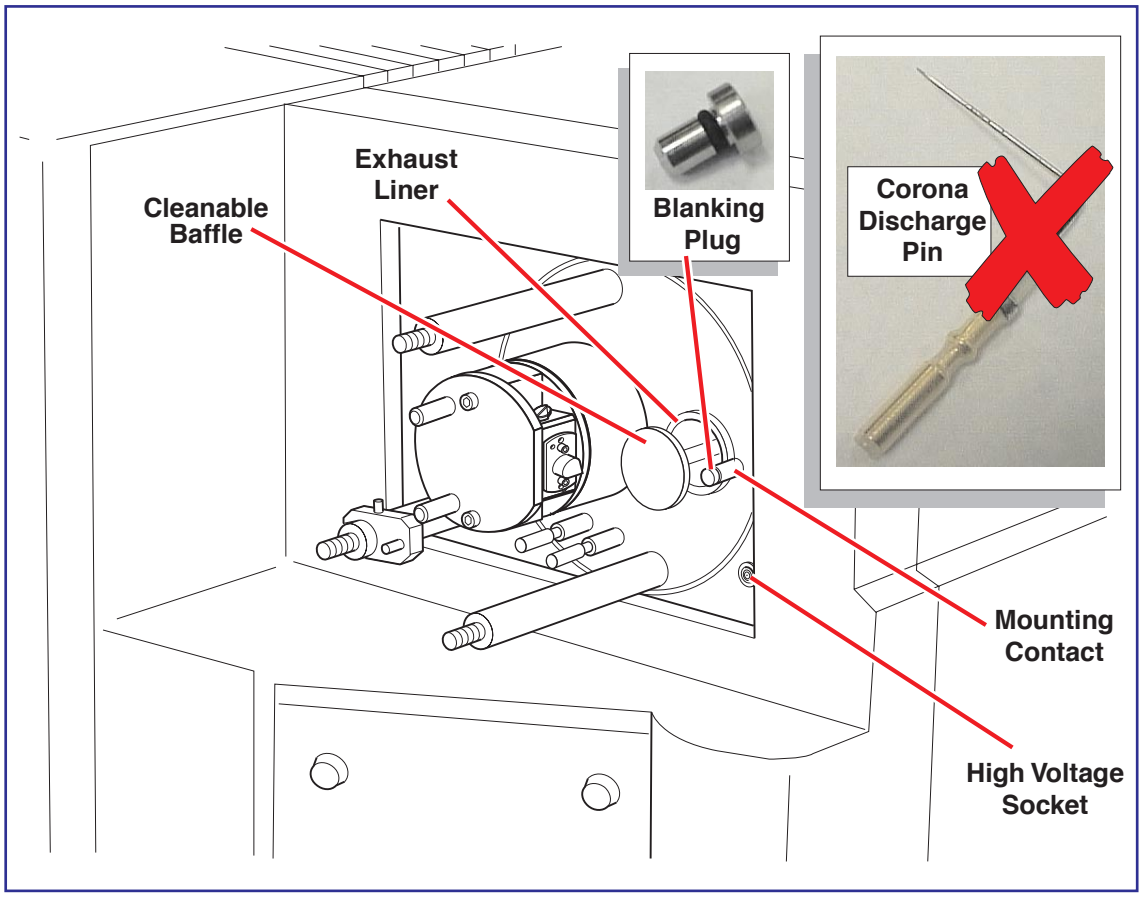
Disconnect the gas and electrical connections from the front panel.

Unscrew the probe thumb nuts and remove the probe

Remove the moulded cover which surrounds the source.

Undo the three thumb screws and remove the probe adjustment flange and glass tube.

Disconnect the APcI high voltage cable from the socket positioned at the bottom right corner of the source flange.



Remove the corona discharge pin from its mounting contact, and fit the blanking plug.

Replace the glass tube, adjustment flange and moulded cover.

Ensure that the source enclosure is in place.

The Z-spray source enclosure consists of the glass tube and the probe adjustment flange.

Connect the source's gas line to **Desolvation Gas** on the front panel. Tighten the nut to ensure a good seal.

Check that the lead of the probe adjustment flange is plugged into the socket labelled **ESI / APcI** on the front panel.

Take the electrospray probe and connect its gas line to **Nebuliser** on the front panel.

Connect the liquid flow of a LC system or syringe pump to the probe.

Insert the probe into the source and tighten the two thumb nuts to secure the probe firmly.

Plug the probe lead into **Capillary / Corona** on the front panel.

On the MassLynx top-level window, click on to launch the tune page.

The top line of the tune page indicates the current ionisation mode.

If necessary, change the ionisation mode using the **Ion Mode** command.

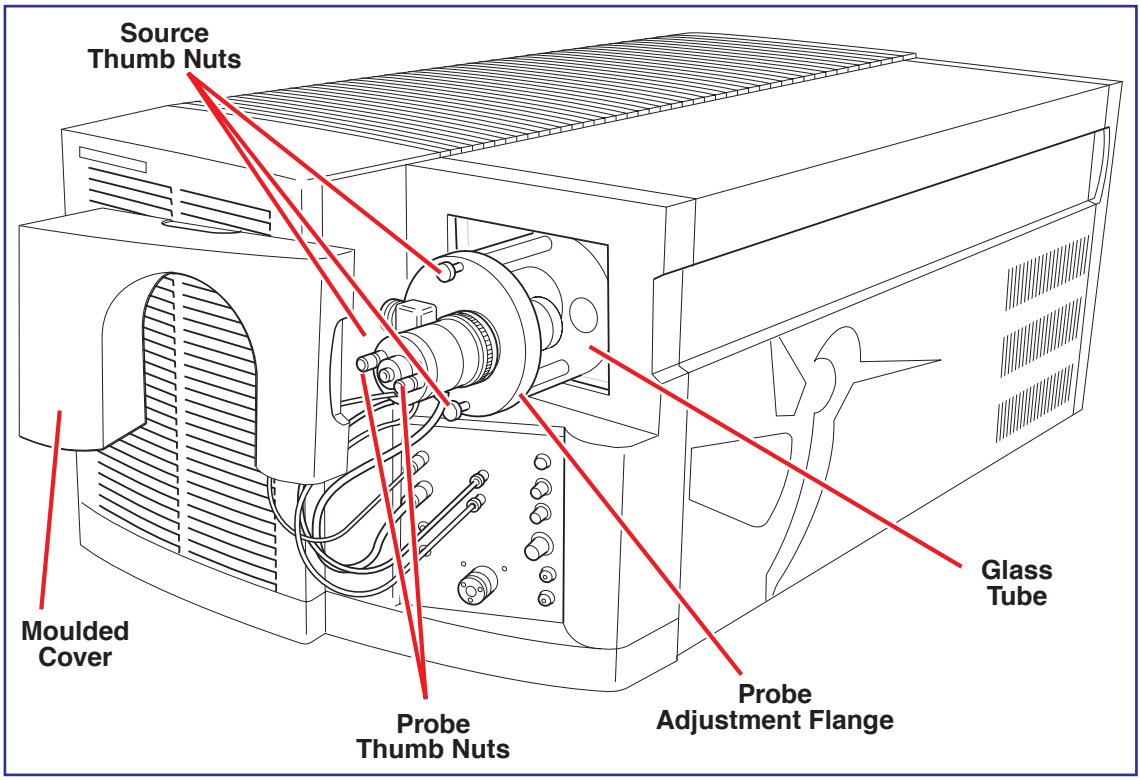
Set **Source Block Temp** to 100°C and **Desolvation Temp** to 20°C.

Warning: Operating the source without the source enclosure will result in solvent vapour escape and the exposure of hot surfaces and high voltages.

Warning: The ion source block can be heated to temperatures of 150°C, and will be maintained at the set temperature when the source enclosure is removed. Touching the ion block when hot may cause burns to the operator.

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Preparation for APcI Operation



If the corona discharge pin is not fitted, proceed as follows:

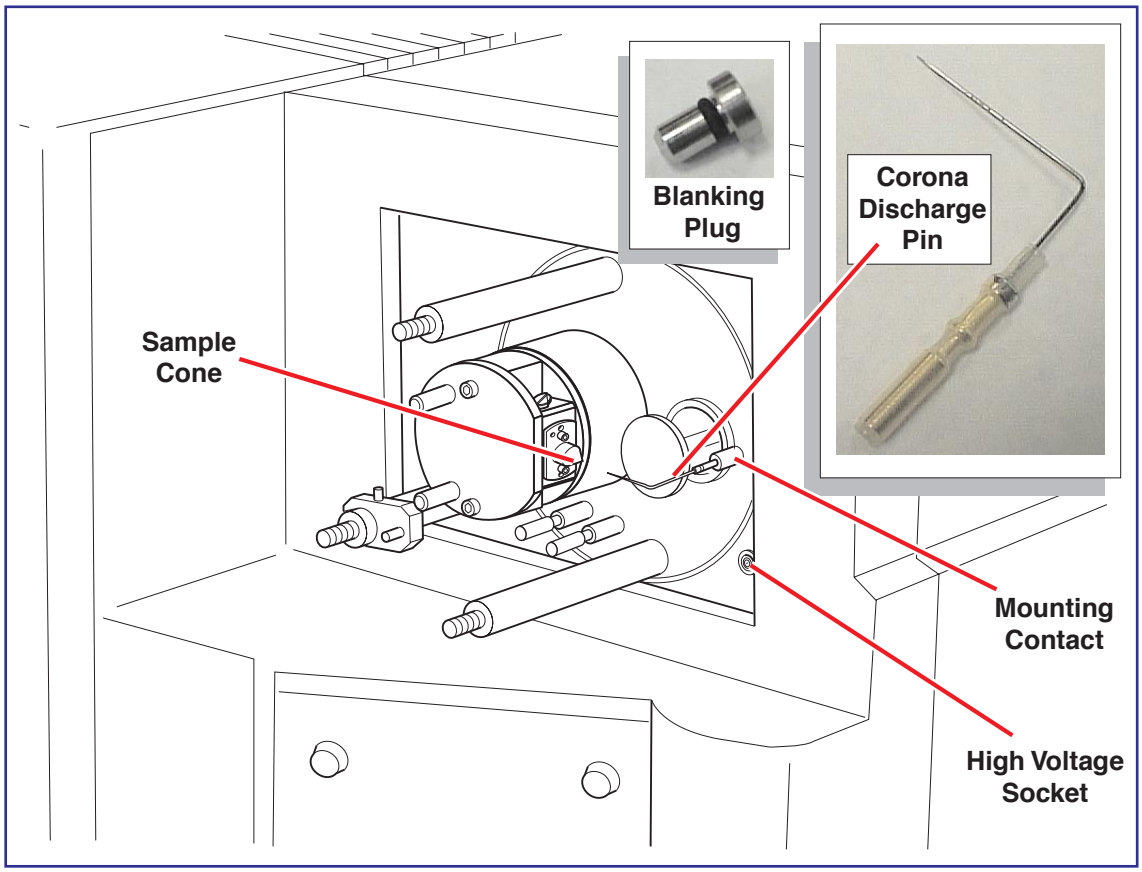
Disconnect the gas and electrical connections from the front panel.

Unscrew the probe thumb nuts and remove the probe.

Remove the moulded cover which surrounds the source.

Undo the three thumb screws and remove the probe adjustment flange and glass tube.

Remove the blanking plug from the discharge pin mounting contact and fit the corona discharge pin, ensuring that the tip is in-line with the tip of the sample cone.



Connect the APcI high voltage cable between **Capillary / Corona** and the socket positioned at the bottom left corner of the source flange.

Replace the glass tube, adjustment flange and moulded cover.

Insert the APcI probe into the source and tighten up the two thumb screws.

On the MassLynx top-level window, click on to launch the tune page.

The top line of the tune page indicates the current ionisation mode.

If necessary, change the ionisation mode using the **Ion Mode** command.

Set **Source Block Temp** to 150°C.

Warning: Operating the source without the source enclosure will result in solvent vapour escape and the exposure of hot surfaces and high voltages. Allow the glass source enclosure to cool after a period of operation at high flow rates before removal.

Warning: The ion source block can be heated to temperatures of 150°C, and will be maintained at the set temperature when the source enclosure is removed. Touching the ion block when hot may cause burns to the operator.

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

The liquid flow should not be started until the gas flow and probe heater are switched on with the probe inserted.

Operate

On the MassLynx top-level window, click on to launch the tune page.

The top line of the tune page indicates the current ionisation mode.

If necessary, change the ionisation mode using the **Ion Mode** command.

Depending on the chosen mode of ionisation, set **Desolvation Temp** or **APcI Probe Temp** to 20°C.

Click on

on the MassLynx tune page.

The instrument will go into the operate mode only if the probe adjustment flange is in place and a probe is inserted.

On the tune page, select **Gas** and **Nitrogen** to turn on the source and probe gases.

Set **Desolvation Gas** to a flow of 150 litres/hour and adjust **Nebuliser** to maximum.

The system is now ready for operation. To obtain an ion beam refer to *Obtaining an Ion Beam* in either the *Electrospray* or the *Atmospheric Pressure Chemical Ionisation* section.

Automatic Pumping and Vacuum Protection

Overview

The instrument is protected against vacuum system faults due to:

- malfunction of the vacuum pumps.
- excessive pressure.
- excessive temperature.

The pump down sequence is fully automated, a command from the data system switching on the rotary pump and turbomolecular pumps simultaneously.

Protection

Transient Pressure Trip

If the vacuum gauge detects a pressure surge above the factory set trip level of 10^{-3} mbar, and if the instrument is in the operate mode, the following events occur:

The critical source, analyser and detector voltages are switched off.

The **Operate** LED shows a steady amber.

The **Vacuum** LED shows a steady amber.

Acquisition will continue, although no mass spectral data are recorded.

When the pressure recovers, the voltages are restored and the **Vacuum** and **Operate** LED's are steady green.

Any further deterioration of the system vacuum results in a pump fault and the system is shut down.

Pump Fault

A pump fault causes the following to occur:

The turbomolecular pumps stop pumping.

On the display the **Vacuum** LED flashes red.

The **Operate** LED is extinguished.

As the turbos slow down the vent valve opens, the rotary pump switches off and the system is vented.

The pumps will not switch on again unless requested to do so.

A pump fault can occur as a result of:

- Over temperature of the turbomolecular pumps.

  If the water cooling fails, then the turbomolecular pumps switch off when their temperature becomes too high.

- Vacuum leak.

  Refer to "Maintenance and Fault Finding" later in this manual.

- Malfunction of the turbomolecular pumps.

  Refer to the pump manufacturer's manuals.

- Malfunction of the rotary pump.

  Refer to the pump manufacturer's manuals.

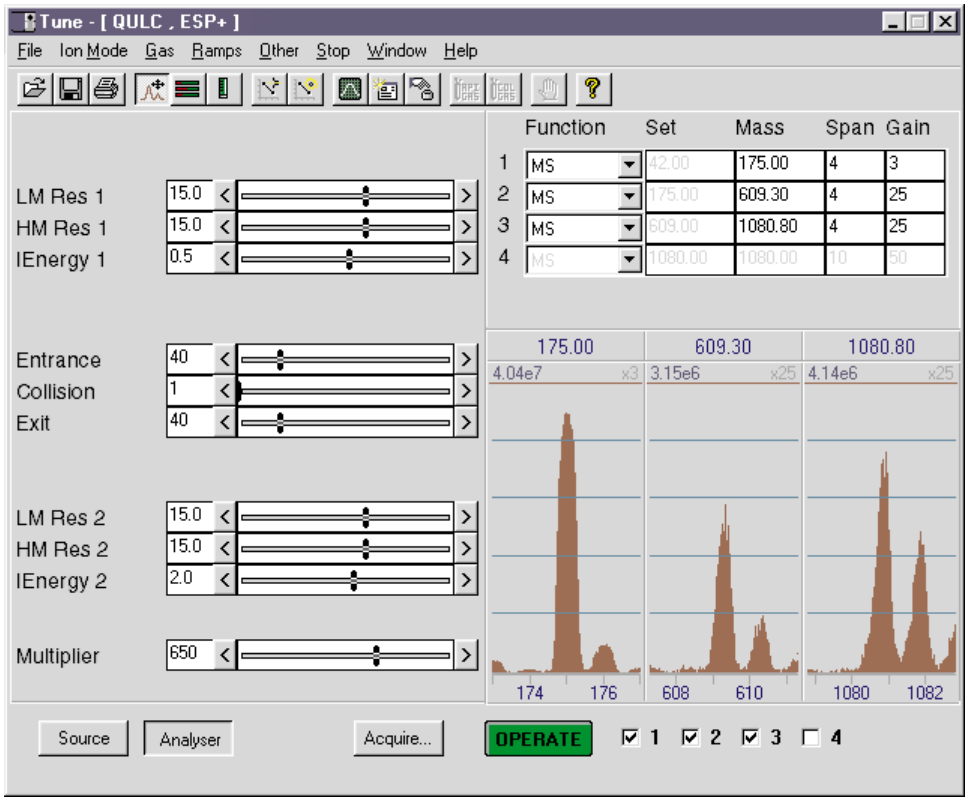
Power Failure

In the event of an unexpected power failure, proceed as follows:

Switch OFF the power to the instrument at the wall mounted isolation switch.

When power is restored, follow the start up procedure as described earlier in this chapter.

Tuning


Tune - [QULC , ESP+]
File Ion Mode Gas Ramps Other Stop Window Help
Function Set Mass Span Gain
1 MS 42.00 175.00 4 3
2 MS 175.00 609.30 4 25
3 MS 609.00 1080.80 4 25
4 MS 1080.00 1080.00 10 50
LM Res 1 15.0
HM Res 1 15.0
IEnergy 1 0.5
Entrance 40
Collision 1
Exit 40
LM Res 2 15.0
HM Res 2 15.0
IEnergy 2 2.0
Multiplier 650
175.00 609.30 1080.80
4.04e7 x3 3.15e6 x25 4.14e6 x25
174 176 608 610 1080 1082
Source Analyser Acquire... OPERATE 1 2 3 4

Before sample data are acquired, the instrument should be tuned and, for the highest mass accuracy, calibrated using a suitable reference compound.

Consult the relevant section of this manual for information concerning source tuning procedures in the chosen mode of operation.

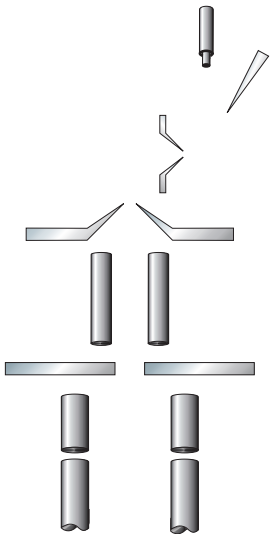
Adjust the tuning parameters in the **Source** and **Analyser** menus to optimise peak shape and intensity at unit mass resolution.

Care should be taken to optimise the value of the collision energy. Note that, in **Daughter** and **Parent** modes, **Collision** and **Exit** are interactive parameters.

Source Voltages

The following illustration shows the various components of Quattro LC's ion optical system. The name in the table's first column is the name used throughout this manual to describe the component. When appropriate, the second column shows the term used in the current MassLynx NT release.

The voltages shown are typical for an instrument in good condition. The polarities given are those actually applied to the electrodes. Only positive values need be entered via the tune page.



	Tune Page Name	ESI		APcI	
		+ve	-ve	+ve	-ve
Electrospray Probe	Capillary	+3.0 (kV)	-3.0 (kV)	Not applicable	
APcI Discharge Pin	Corona	Not applicable		+3.0 (kV)	-2.0 (kV)
Sample Cone	Cone	+25 (V)	-25 (V)	+25 (V)	-25 (V)
Extraction Cone	Extractor	+3 (V)	-3 (V)	+3 (V)	-3 (V)
Hexapole Transfer Lens	RF Lens	+0.5 (V)	-0.5 (V)	+0.5 (V)	-0.5 (V)
Differential Aperture		Not adjustable (ground)			
Prefilter		Not applicable			
Quadrupole Analyser		Not applicable			

Calibration

Information concerning the calibration of Quattro LC is provided in *Mass Calibration* later in this document and in the *Guide to Data Acquisition*.

Data Acquisition

The mechanics of the acquisition of sample data are comprehensively described in the *Guide to Data Acquisition*. Refer to that publication for full details.

Data Processing

The processing of sample data is comprehensively described in the *MassLynx NT User's Guide*. Refer to that publication for full details.

Setting Up for MS-MS Operation

The following notes provide a worked example for the acquisition of daughter ion data. The experiment is performed in the ESI positive mode using reserpine as a model analyte. Reserpine, admitted by constant infusion at a concentration of 50 pg/µl, provides a stable and persistent source of ions for instrument tuning in both the MS and MS-MS modes of operation.

The basic sequence of events is as follows:

- Tuning MS1 (described earlier in this chapter).
- Tuning MS2 (described earlier in this chapter).
- Parent ion selection.
- Fragmentation.

Parent Ion Selection

For maximum sensitivity in daughter ion analysis the centre of the parent ion selected by MS1 must be accurately found.

The nominal mass of the parent is first determined (if unknown) by viewing it in MS mode:

Set up a 1 box display in the tune page and set **Function** to **MS**. Observe the candidate parent ion in the tune display and determine its nominal mass.

In this example the reserpine ion at m/z 609 will be used as a model parent.

The accurate top of the parent ion can be found experimentally by performing a "daughter ion scan" over a restricted mass range in the absence of collision gas.

On the tune page place the mouse cursor on the **Set** mass for peak 2 and type in the nominal mass of the parent ion selected by MS1, in this case 609.

Double click on peak 2 to zoom in.

Select **Other** followed by **Scope Parameters**.

Set **Mass Increment** to 0.1m/z.

Using the left and right arrow controls, vary the set **Mass** value between 608.5 and 609.5 while observing the intensity of the non-fragmented parent ion in the tune display.

Adjust the **Set** mass in the same manner to optimise the intensity of the parent.

Fragmentation

Set up a wide range daughter ion scan by adjusting the **Mass** and **Span** parameters for peak 2.

At this point, with the collision gas off, a few daughter ions of low intensity may be visible. These are the products of unimolecular dissociations.

Argon (99.9% pure) is recommended as the collision gas.

Select **Gas** and turn on **Collision**.

Adjust **CID Gas** on the front panel to admit sufficient gas to attenuate the parent ion peak by about 50%.

Adjust the **Entrance, Collision** and **Exit** parameters in the **Analyser** menu to produce the desired degree of fragmentation. (These parameters are interactive in MS-MS operation.)

In daughter ion analysis maximum transmission (sensitivity) can be achieved by the following adjustments:

- Increasing **RF Lens** on the **Source** tune window.
- Increasing **IEnergy 1** on the **Analyser** window.
- Optimising **Collision**.
- Optimising **Exit**.
- Optimising **Entrance**.
- Optimising the collision gas pressure using the **CID Gas** needle valve.

Additionally, transmission can be improved at the expense of specificity by reducing **HM Res** on the **Analyser** window. In most cases, where chemical interference with the parent ion is not acute, the loss of specificity is negligible.

Shutdown Procedures

Emergency Shutdown

In the event of having to shut down the instrument in an emergency, proceed as follows:

Switch **OFF** the power at the wall mounted isolation switch(es), if fitted. If not, switch the power off at the rear of the instrument and switch off all peripherals.

Isolate any LC systems to prevent solvent flowing into the source.

A loss of data is likely.

Overnight Shutdown

When the instrument is to be left unattended for any length of time, for example overnight or at weekends, proceed as follows:

Switch off the LC pumps.

On the MassLynx top-level window, click on to launch the tune page.

Click on

.

This will change from green to grey indicating that the instrument is no longer in operate mode.

Undo the finger-tight connector on the probe to release the tubing leading from the LC system.

Before disconnecting the probe, it is good practice to temporarily remove the probe and flush it of any salts, buffers or acids.

If APcI is being used, switch off the probe heater or reduce it to ambient temperature.

Caution: Leaving the APcI probe hot with no gas or liquid flow will shorten the lifetime of the probe heater.

Select **Gas** followed by **Nitrogen** to turn off the supply of nitrogen gas.

If the instrument is not to be used for a long period of time:

Reduce **Source Block Temp** to 60°C.

Complete Shutdown

If the instrument is to be left unattended for extended periods, proceed as follows:

Switch off the LC pumps.

On the MassLynx top-level window, click on to launch the tune page.

Click on

on the tune page.

This will change from green to grey indicating that the instrument is no longer in operate mode.

Undo the finger-tight connector on the probe to release the tubing leading from the LC system.

Before disconnecting the probe, it is good practice to temporarily remove the probe and flush it of any salts, buffers or acids.

If APcI is being used, switch off the probe heater or reduce it to ambient temperature.

Caution: Leaving the APcI probe hot with no gas or liquid flow will shorten the lifetime of the probe heater.

Select **Gas** followed by **Nitrogen** to turn off the supply of nitrogen gas.

Select **Other** from the menu bar at the top of the tune page. Click on **Vent**.

The rotary pump and the turbomolecular pumps switch off. When the turbomolecular pumps have run down to half of their normal operating speed the vent valve opens and the instrument is vented to atmosphere.

Exit MassLynx.

Shut down the computer.

Switch off all peripherals.

Switch off the power to the instrument using the switch on the rear panel of the instrument.

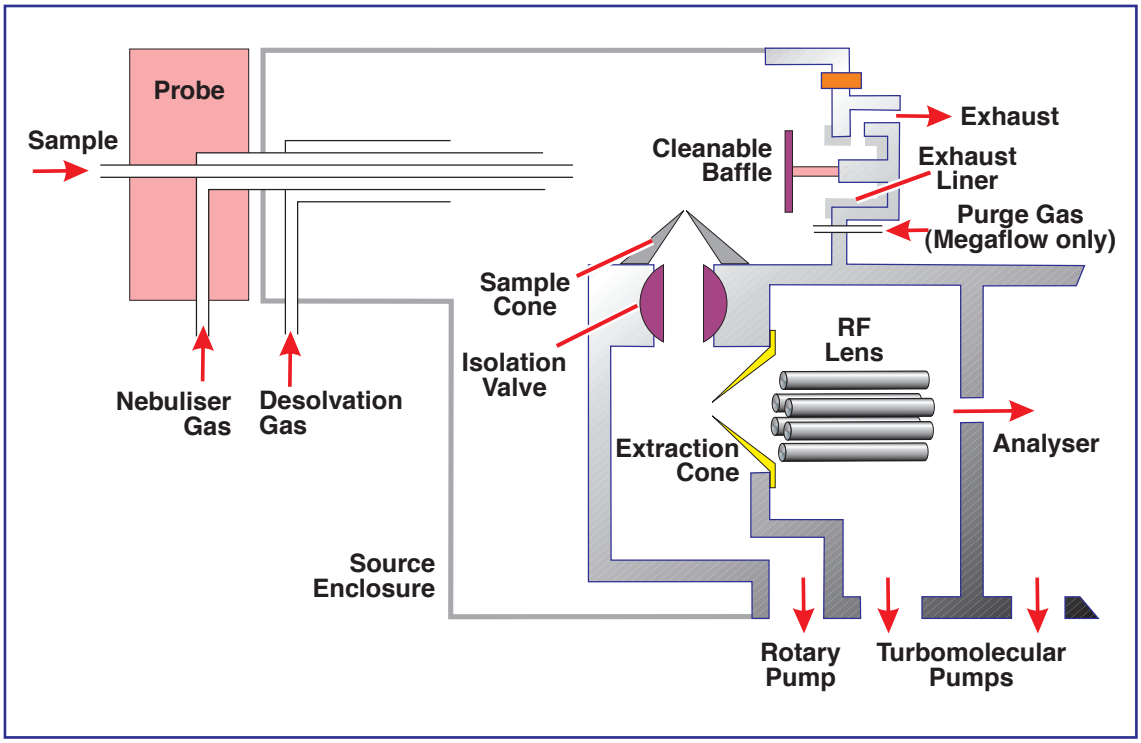
Switch off power at the wall mounted isolation switches.

If the instrument is to be switched off for more than one week:

Drain the oil from the rotary pump according to the manufacturer's instructions.

Electrospray

Introduction



The ESI interface consists of the standard Z-spray source fitted with an electrospray probe. See the following chapter for information concerning the optional nanoflow interface.

Mobile phase from the LC column or infusion pump enters through the probe and is pneumatically converted to an electrostatically charged aerosol spray. The solvent is evaporated from the spray by means of the desolvation heater. The resulting analyte and solvent ions are then drawn through the sample cone aperture into the ion block, from where they are then extracted into the analyser.

The electrospray ionisation technique allows rapid, accurate and sensitive analysis of a wide range of analytes from low molecular weight (less than 200 Da) polar compounds to biopolymers larger than 100 kDa.

Generally, compounds of less than 1000 Da produce singly charged protonated molecules ($[M+H]^+$) in positive ion mode. Likewise, these low molecular weight analytes yield ($[M-H]^-$) ions in negative ion mode, although this is dependent upon compound structure.

High mass biopolymers, for example peptides, proteins and oligonucleotides, produce a series of multiply charged ions. The acquired data can be transformed by the data system to give a molecular weight profile of the biopolymer.

The source can be tuned to fragment ions within the ion block. This can provide valuable structural information for low molecular weight analytes.

The most common methods of delivering sample to the electrospray source are:

- **Syringe pump and injection valve.**

  A flow of mobile phase solvent passes through an injection valve to the electrospray source. This is continuous until the pump syringes empty and need to be refilled. Sample is introduced through the valve injection loop (usually 10 or 20µl capacity) switching the sample plug into the mobile phase flow. Tuning and acquisition are carried out as the sample plug enters the source. (At a flow rate of 10 µl/min a 20µl injection lasts 2 minutes.)

- **Reciprocating pump and injection valve.**

  A flow of mobile phase solvent passes through an injection valve to the electrospray source. Sample injection and analysis procedure is the same as for the syringe pump. The pump reservoirs are simply topped up for continuous operation. The most suitable reciprocating pumps for this purpose are those which are specified to deliver a flow between 1 µl/min and 1 ml/min. A constant flow at such rates is more important than the actual flow rate. The injection valve on reciprocating pumps may be replaced by an autosampler for unattended, overnight operation.

- **Infusion pump.**

  The pump syringe is filled with sample in solution. The infusion pump then delivers the contents of the syringe to the source at a constant flow rate. This arrangement allows optimisation and analysis while the sample flows to the source at typically 5-30 µl/min. Further samples require the syringe to be removed, washed, refilled with the next sample, and replumbed.

A 50:50 mixture of acetonitrile and water is a suitable mobile phase for the syringe pump system and the reciprocating pump systems. This is appropriate for positive and negative ion operation.

> *Positive ion operation may be enhanced by 0.1 to 1% formic acid in the sample solution.*
>
> *Negative ion operation may be enhanced by 0.1 to 1% ammonia in the sample solution. Acid should not be added in this mode.*
>
> *These additives should not be used for flow injection analysis (FIA) studies, to allow easy change over between positive and negative ion analysis.*

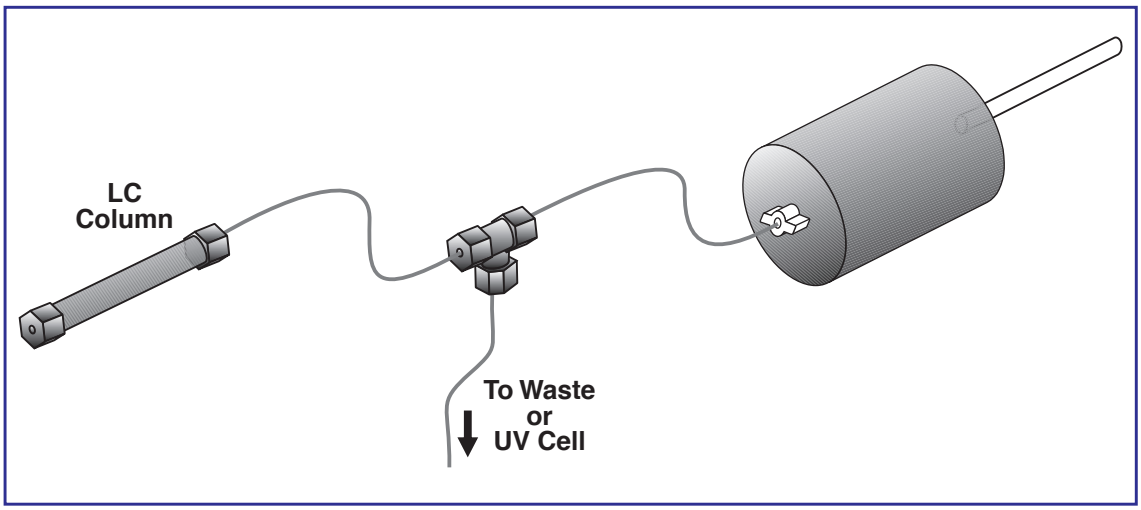
Degassed solvents are recommended for the syringe and reciprocating pumps. Degassing can be achieved by sonification or helium sparging. The solvents should be filtered, and stored under cover at all times.

It is wise periodically to check the flow rate from the solvent delivery system. This can be carried out by filling a syringe barrel or a graduated glass capillary with the liquid emerging from the probe tip and timing a known volume, say 10μl. Once the rate has been measured and set, a note should be made of the back pressure readout on the pump as fluctuation of this reading can indicate problems with the solvent flow.

Post-column Splitting

Although the electrospray source can accommodate flow rates up to 1 ml/min, it is recommended that the flow is split post-column to approximately 200 μl/min. Also, even at lower flow rates, a split may be required for saving valuable samples.

The post-column split consists of a zero dead-volume tee piece connected as shown.



The split ratio is adjusted by increasing or decreasing the back pressure created in the waste line, by changing either the length or the diameter of the waste tube. A UV cell may also be incorporated in the waste line, avoiding the requirement for in-line, low volume “Z cells”. As the back pressure is varied, the flow rate at the probe tip should be checked as described above.

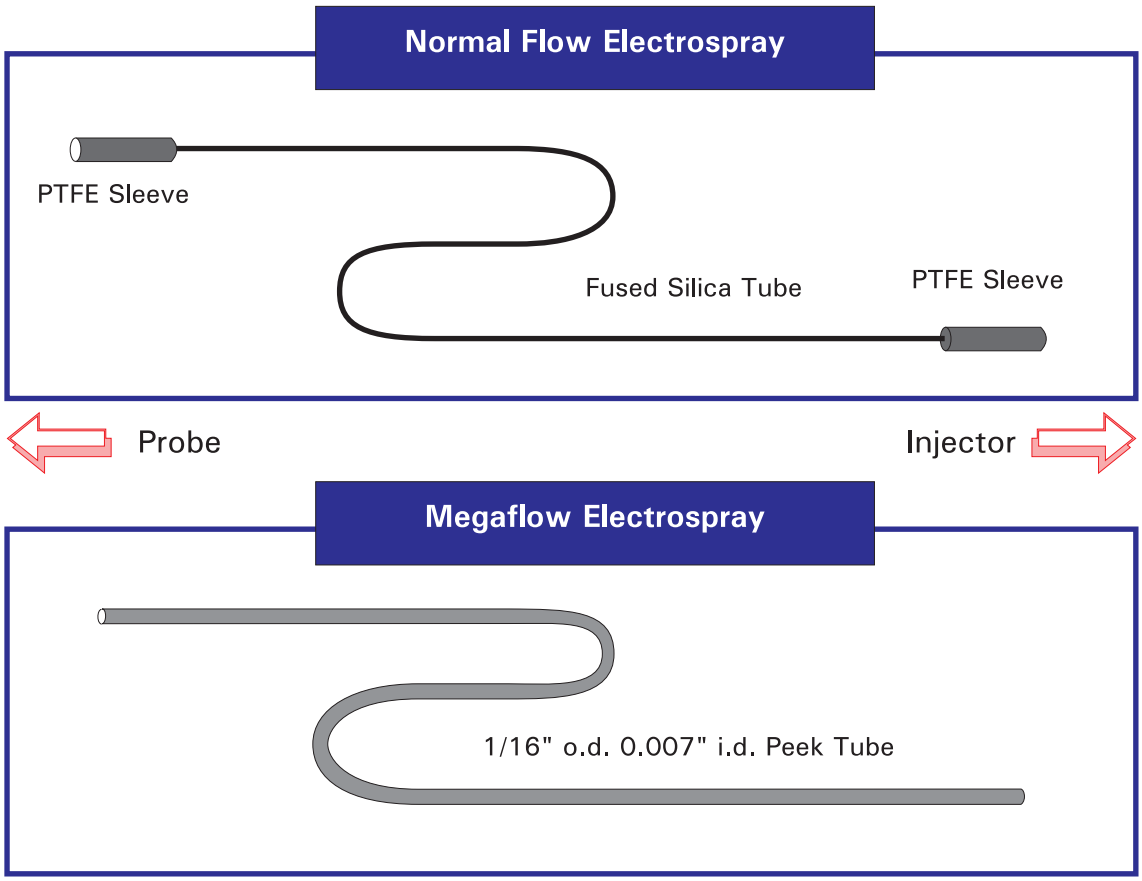
These principles apply to splitting for both megaflow and normal flow electrospray.

Megaflow

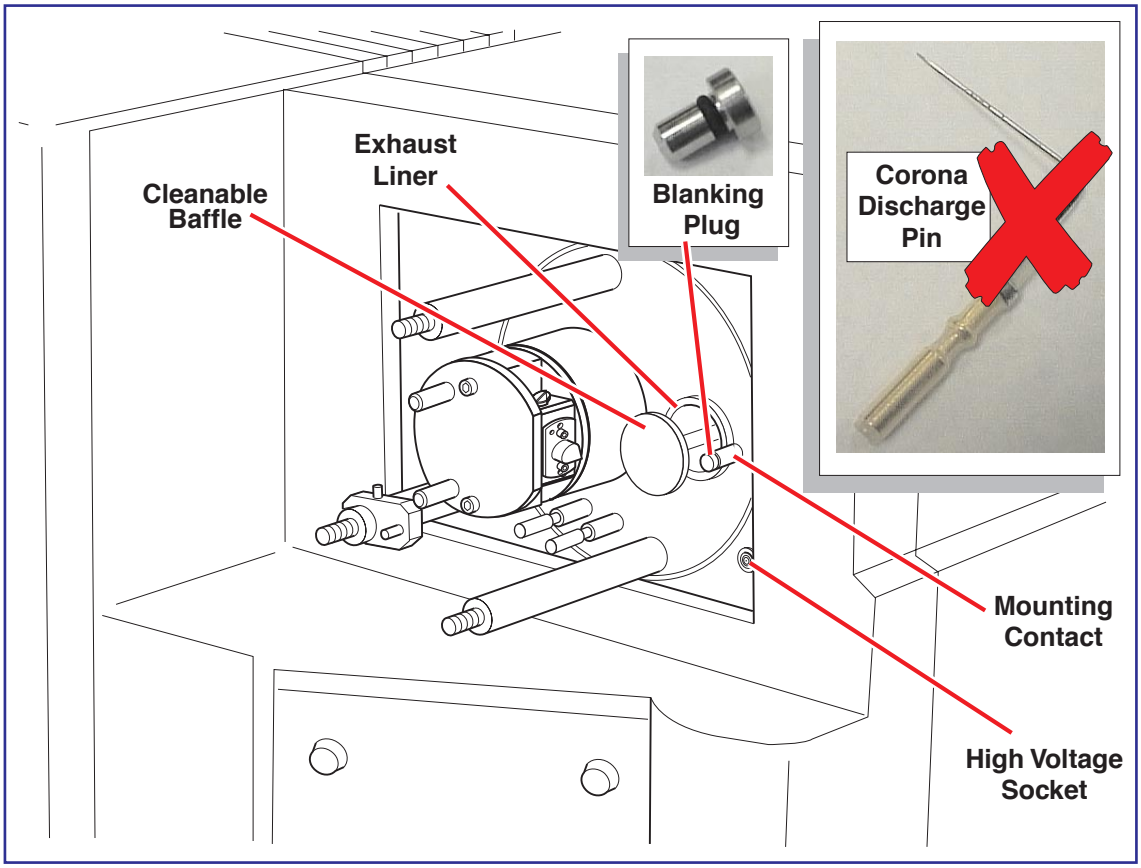
Megaflow electrospray enables flow rates from 200 µl/min to 1 ml/min to be accommodated. This allows Microbore (2.1mm) or 4.6mm diameter columns to be interfaced without splitting.

Changing Between Flow Modes

When changing between megaflow and standard electrospray operation, it is essential that the correct tubing is used to connect the probe to the sample injector. For megaflow operation $^{1}/_{16}$" o.d., 0.007" i.d. peek tubing, easily identified by its yellow stripe, is used. This replaces the standard fused silica tube, together with the PTFE sleeves.



Operation



Ensure that the source is assembled as described in *Maintenance and Fault Finding*, and that the instrument is pumped down and prepared for electrospray operation as described in *Routine Procedures*.

Ensure that a supply of nitrogen has been connected to the gas inlet at the rear of the instrument and that the head pressure is between 6 and 7 bar (90-100 psi).

Ensure that the exhaust liner and the cleanable baffle are fitted to the source.

This is important for optimum electrospray intensity and stability when operating at low flow rates.

Checking the ESI Probe

Connect the electrospray probe to a pulse free pump.

Solvent should be degassed to prevent beam instabilities caused by bubbles.

Connect the PTFE tubing of the electrospray probe to **Nebulising Gas** on the front panel. Secure with the nut provided.

With the probe removed from the source turn on the liquid flow at 10 µl/min and check that liquid flow is observed at the tip of the capillary.

To avoid unwanted capillary action effects, do not allow liquid to flow to the probe for long periods without the nitrogen switched on.

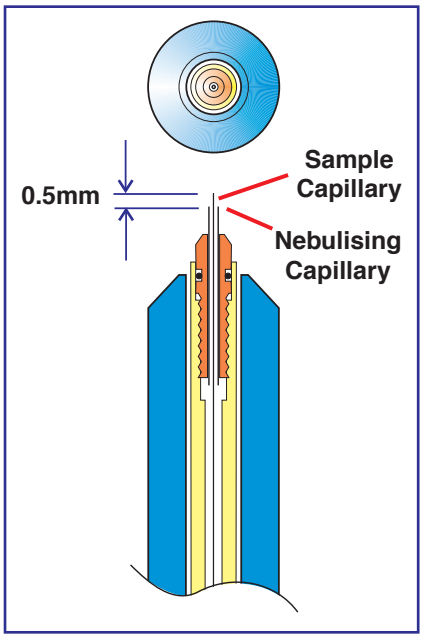
Turn on **Nitrogen** by selecting **Gas**, and check that a nebuliser flow of less than 100 litres/hour is registered.

To monitor the flow rate, select **Window** *then* **Gas Flow** *on the tune page and observe the readback window.*

Check that there is gas flow at the probe tip and ensure that there is no significant leakage of nitrogen elsewhere.

Adjust the probe tip to ensure complete nebulisation of the liquid.

There should be approximately 0.5 mm of sample capillary protruding from the nebulising capillary.



The tip of the electrospray probe can influence the intensity and stability of the ion beam. A damaged or incorrectly adjusted probe tip will lead to poor electrospray performance.

Using a magnifying glass ensure that both inner and outer stainless steel capillaries are straight and circular in cross-section.

Ensure that the inner stainless steel capillary is coaxial to the outer capillary.

If the two capillaries are not coaxial, it is possible to bend the outer capillary slightly using thumbnail pressure.

Insert the probe into the source and tighten the two thumb screws.

Plug the probe high voltage cable into **Capillary / Corona** on the front panel.

Obtaining an Ion Beam

If necessary, change the ionisation mode using the **Ion Mode** command.

The top line of the tune page indicates the current ionisation mode.

Using the needle valve on the front panel, set the desolvation gas flow rate to 300 litres/hour.

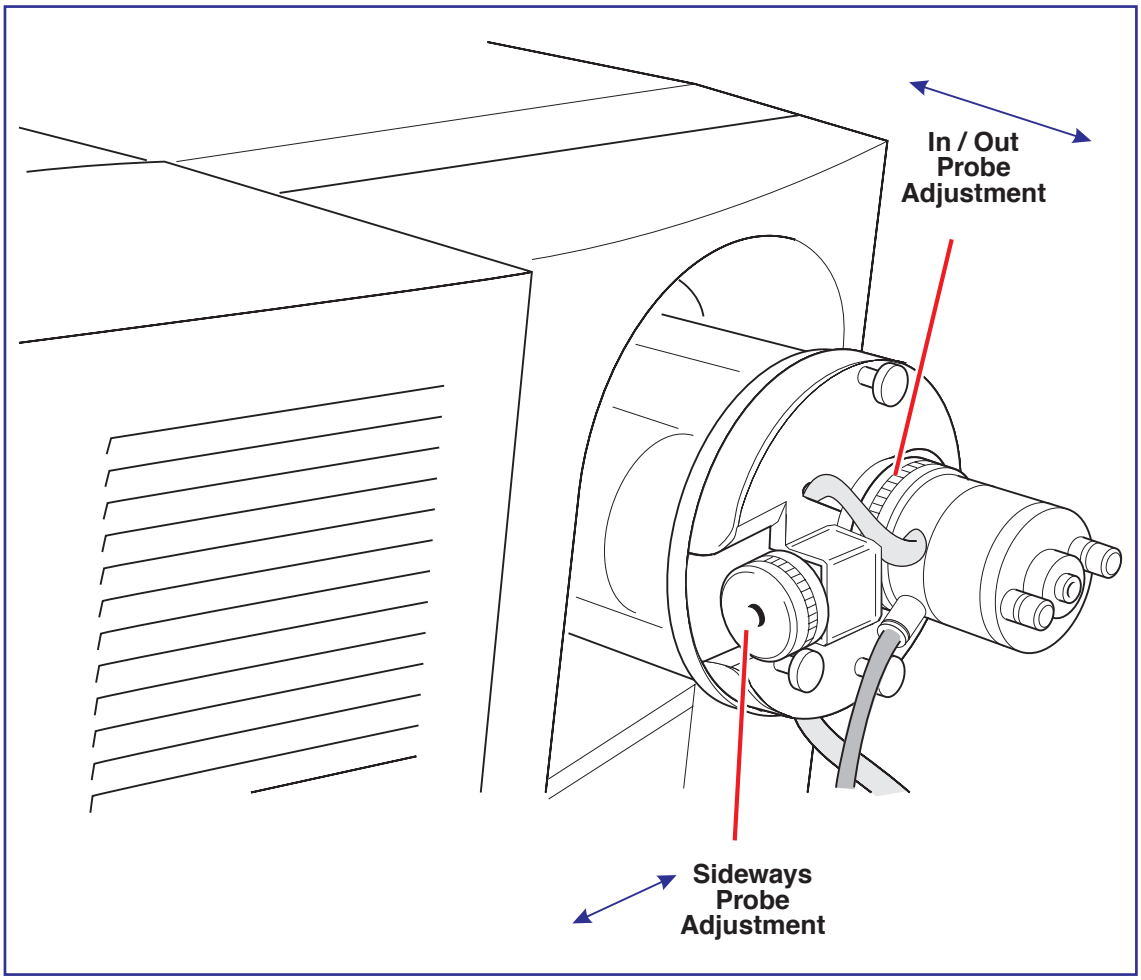
To monitor the flow rate, select **Window** *then* **Gas Flow** *on the tune page and observe the readback window.*

Turn on the liquid flow at 10 µl/min and set **Desolvation Temp** to 100°C.

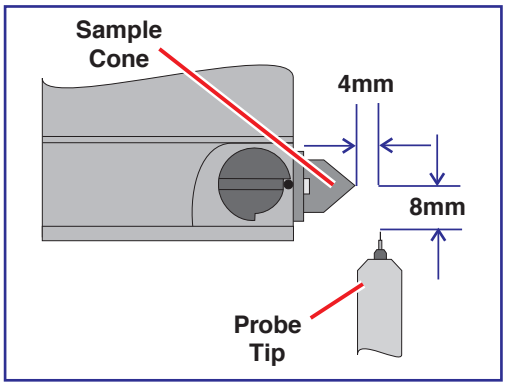
Tuning and Optimisation

The following parameters, after initial tuning, should be optimised using a sample representative of the analyte to be studied. It will usually be found, with the exception of the sample cone voltage, that settings will vary little from one analyte to another.

Probe Position



The position of the probe is adjusted using the probe adjustment collar (in/out) and the adjustment knob (sideways) located to the left of the probe. The two screws can be adjusted singly or simultaneously to optimise the beam. The position for optimum sensitivity and stability for low flow rate work (10 µl/min) is shown.

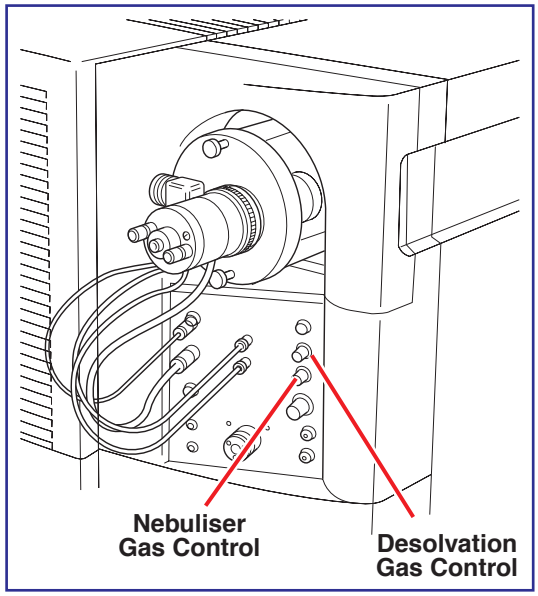


Small improvements may be gained by varying the position using the sample and solvent system under investigation. The following information should be considered when setting the probe position:

- 10mm of movement is provided in each direction, with 1.25mm of travel per revolution of the probe positioning controls.
- At higher liquid flow rates the probe tip should be positioned further away from the sample cone to achieve optimum stability and sensitivity. The position is less critical than at lower flow rates.

Nebuliser Gas

Optimum nebulisation for electrospray performance is achieved with a nitrogen flow between 70 and 90 litres per hour. This can be achieved by fully opening the **Nebuliser** flow control valve, which is situated on the instrument's front panel.



Desolvation Gas

The desolvation gas, also nitrogen, is heated and delivered as a coaxial sheath to the nebulised liquid spray by the desolvation nozzle.

The position of the desolvation nozzle heater is fixed relative to the probe tip and requires no adjustment.

The **Desolvation Gas** flow rate is adjusted by the control value situated on the instrument's front panel. The optimum **Desolvation Temp** and flow rate is dependent on mobile phase composition and flow rate. A guide to suitable settings is given below.

To monitor the flow rate, select **Window** *then* **Gas Flow** *on the tune page and observe the readback window. The* **Desolvation Gas** *flow rate indicated on the MassLynx tune page represents total drying flow, that is desolvation gas + cone gas (nanoflow only) + purge gas (if enabled).*

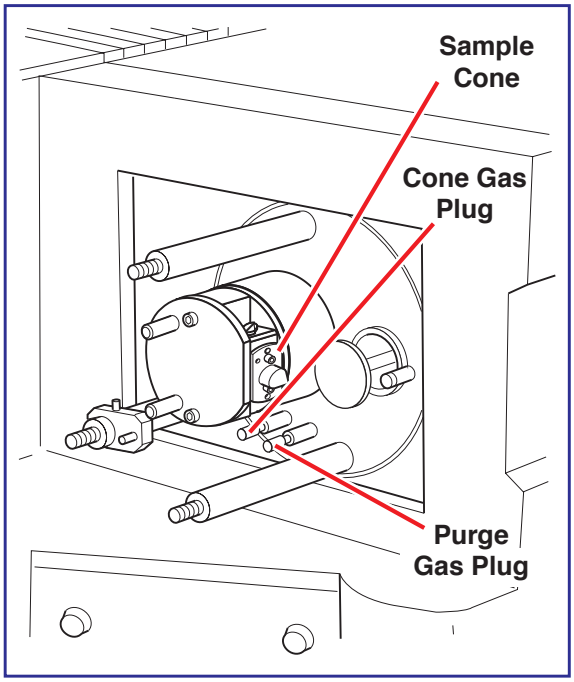
Solvent Flow Rate µl/min	**Desolvation Temp** °C	**Desolvation Gas Flow Rate** litres/hour
<10	100 to 120	200 to 250
10 to 20	120 to 250	200 to 400
20 to 50	250 to 350	200 to 400
>50	350 to 400	500 to 750

Higher desolvation temperatures gives increased sensitivity. However increasing the temperature above the range suggested reduces beam stability. Increasing the gas flow rate higher than the quoted values leads to unnecessarily high nitrogen consumption.

Caution: Do not operate the desolvation heater for long periods of time without a gas flow. To do so could damage the source.

Cone Gas

The cone gas should be used only in the nanoflow mode (see the following chapter). Ensure that the cone gas outlet, situated inside the source enclosure, is blanked for normal ESI operation.

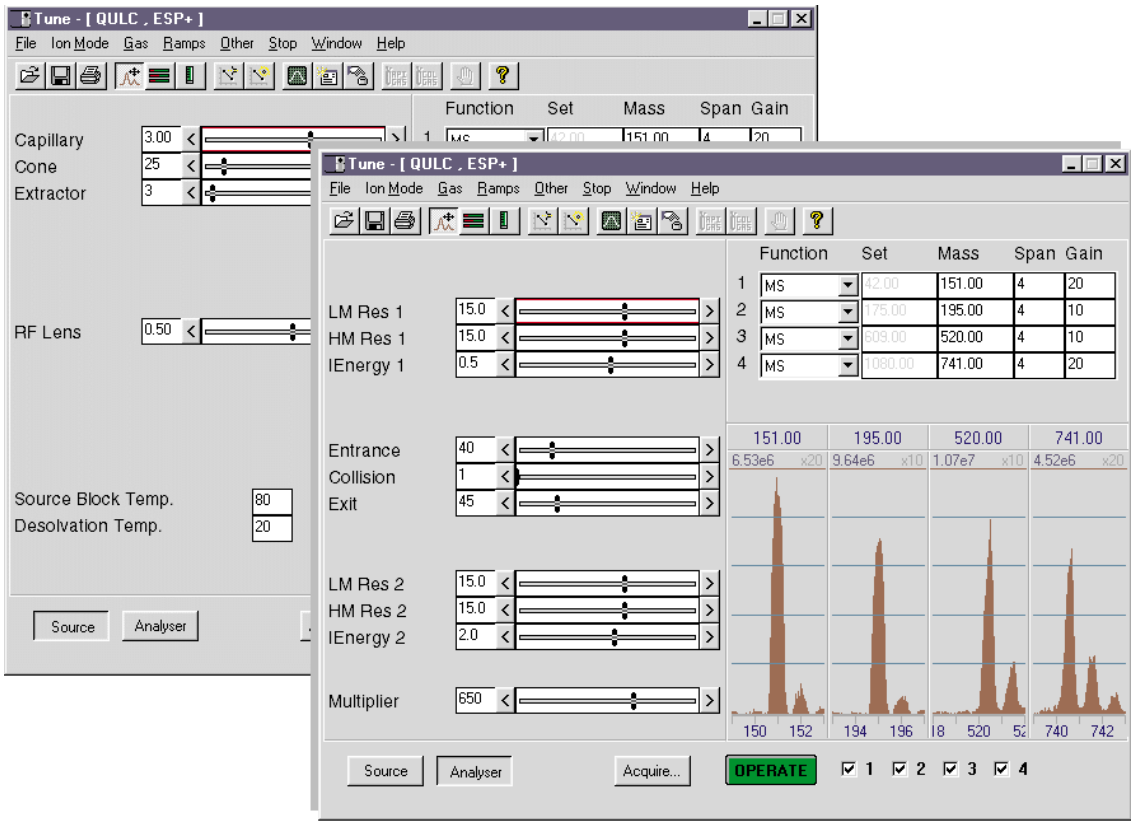


Purge Gas

The purge gas is not necessary for most ESI applications. It may be useful for megaflow operation where an analyte is susceptible to acetonitrile adducting.

Purge gas is enabled simply by removing the blanking plug from the outlet situated within the source enclosure.

Purge gas flow rate is a constant fraction (30%) of the total desolvation gas flow.



Source temperature

100°C is typical for 50:50 CH_3CN:H_2O at solvent flow rates up to 50 µl/min. Higher source temperatures, up to 150°C, are necessary for solvents at higher flow rates and higher water content.

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Capillary Voltage

Capillary usually optimises at 3.0kV, although some samples may tune at values above or below this, within the range 2.5 to 4.0kV for positive electrospray. For negative ion operation a lower voltage is necessary, typically between 2.0 and 3.5kV.

At high flow rates this parameter may optimise at a value as low as 1kV.

Sample Cone Voltage

A **Cone** setting between 25V and 70V will produce ions for most samples, although solvent ions prefer the lower end and proteins the higher end of this range. Whenever sample quantity and time permit, **Cone** should be optimised for maximum sensitivity, within the range 15V to 150V.

Extraction Cone Voltage

Extractor optimises at 3 to 10V for most samples, and all samples may be optimised within the range 0 to 20V. Higher values than this may be set to induce ion fragmentation of low molecular weight samples. Increasing **Extractor** and **Cone** together will give rise to more severe fragmentation conditions.

Low Mass Resolution and High Mass Resolution

Peak width is affected by the values of low mass resolution (**LM Res**) and high mass resolution (**HM Res**). Both values should be set low (typically 5.0) at the outset of tuning and only increased for appropriate resolution after all other tuning parameters have been optimised. A value of 15 (arbitrary units) usually gives unit mass resolution on a singly charged peak up to m/z 1600.

Ion Energy

The ion energy parameter usually optimises in the range 0V to 3V. It is recommended that the value is kept as low (or negative) as possible without reducing the height intensity of the peak. This will help obtain optimum resolution.

If, in positive ion mode, an ion energy value below -1V can be used without reducing the peak intensity then source cleaning is recommended.

Megaflow Hints

With this high flow rate technique the setup procedure involves making the following adjustments:

- increase **Drying Gas** flow to approximately 750 litres/hour.
- increase **Desolvation Temp** to 400°C.
- increase **Source Block Temp** to 150°C.
- move the probe further away from the sample cone.

When changing from electrospray to megaflow operation it is not necessary to adjust any source voltages.

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Cluster ions are rarely observed with Z-spray. However solvent droplets may form within the source enclosure if the source and desolvation temperatures are too low.

Refer to the previous section on operating parameters for typical desolvation gas flow rates.

Purge gas can be used during megaflow operation to stop the source enclosure from overheating. This is also beneficial when the analyte is susceptible to acetonitrile adducting. Purge gas is enabled by removing the blanking plug from the outlet situated within the source enclosure.

If the sample is contained within a 'dirty matrix' the probe may be moved away from the sample cone to extend time between source cleaning operations. This may incur a small loss in sensitivity.

Warning: It is normal for the source enclosure, the glass tube and parts of the probe mounting flange, to get hot during prolonged megaflow operation. Care should be taken when handling source components during and immediately after operation.

The source enclosure will run cooler if purge gas is used.

Warning: For health and safety reasons always ensure the exhaust line is vented outside the building or to a fume hood.

Warning: Ensure that a plastic bottle is connected in the exhaust line to collect any condensed solvents.

Removing the Probe

To remove the probe from the source proceed as follows:

On the tune page deselect OPERATE.

Switch off the liquid flow and disconnect from the probe.

Select **Gas** and turn off **Nitrogen**.

Disconnect the probe cable from the instrument.

Disconnect the nebulising gas supply from the instrument.

Sample Analysis and Calibration

General Information

Care should be taken to ensure that samples are fully dissolved in a suitable solvent. Any particulates must be filtered to avoid blockage of the transfer line or the probe's capillary. A centrifuge can often be used to separate solid particles from the sample liquid.

There is usually no benefit in using concentrations greater than 20 pmol/µl for biopolymers or 10 ng/µl for low molecular weight compounds.

Higher concentrations will not usually improve analytical performance. Conversely, for biopolymers, lower concentrations often yield better electrospray results. Higher levels require more frequent source cleaning and risk blocking the transfer capillary.

Optimisation for low molecular weight compounds may usually be achieved using a concentration of 1 ng/μl.

Samples with phosphate buffers and high levels of salts should be avoided. Alternatively, at the expense of a small drop in sensitivity, the probe can be pulled away from the sample cone to minimise the deposit of involatile material on the cone.

To gain experience in sample analysis, it is advisable to start with the qualitative analysis of known standards. A good example of a high molecular weight sample is horse heart myoglobin (molecular weight 16951.48) which produces a series of multiply charged ions that can be used to calibrate the m/z scale from 800-1600 in either positive ion or negative ion mode.

Polyethylene glycol mixtures, for example 300/600/1000, are low molecular weight samples suitable for calibrating the m/z scale from approximately 100 to 1200 in positive ion mode. A mixture of sugars covers the same range in negative ion mode.

Alternatively, a mixture of sodium iodide and caesium iodide (or a mixture of sodium iodide and rubidium iodide) can be used for calibration.

Detailed information on data acquisition and processing can be found in the *MassLynx NT User's Guide*. Detailed information on mass calibration can be found in *Mass Calibration* later in this document.

Typical ES Positive Ion Samples

- Peptides and proteins.
- Small polar compounds.
- Drugs and their metabolites.
- Environmental contaminants (e.g. pesticides / pollutants).
- Dye compounds.
- Some organometallics.
- Small saccharides.

Typical ES Negative Ion Samples

- Some proteins.
- Some drug metabolites (e.g. glucuronide conjugates).
- Oligonucleotides.
- Some saccharides and polysaccharides.

Chromatographic Interfacing

Electrospray ionisation can be routinely interfaced to reversed phase and normal phase chromatographic separations. Depending on the LC pumping system, chromatography column and setup, there are some basic options:

- Microbore and capillary chromatography separations employing 1 mm diameter (and smaller) columns can be interfaced directly to the electrospray probe. Typical flow rates for such columns may be in the region of 3-50 µl/min. It is suggested that a syringe pump is used to deliver these constant low flow rates through a capillary column. Alternatively, accurate pre-column splitting of higher flow rates from reciprocating pumps can be investigated.

  In all cases, efficient solvent mixing is necessary for gradient elution separations. This is of paramount importance with regard to low flow rates encountered with capillary columns. HPLC pump manufacturers' recommendations should be heeded.

- 2.1mm diameter reversed phase columns are gaining popularity for many separations previously addressed by 4.6mm columns. Typically flow rates of 200 µl/min are used, allowing direct coupling to the electrospray source. The increased sample flow rate requires increased source temperature and drying gas flow rate.

  A UV detector may be placed in-line to the Quattro LC probe. However, ensure that the volume of the detector will not significantly reduce the chromatographic resolution. Whenever a UV detector is used, the analog output may be input to MassLynx NT for chromatographic processing.

- The interfacing of 4.6mm columns to the electrospray source can be achieved either by flow splitting or by direct coupling. In both cases an elevated source temperature and drying gas flow rate are required. In general, the best results are obtained by splitting after the column using a zero dead volume tee piece so that 200-300 µl/min is transferred to the source.

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Conventional reverse phase and normal phase solvent systems are appropriate for LC-electrospray.

Involatile buffers may be used but prolonged periods of operation are not recommended. When using involatile buffers the probe should be moved as far away from the sample cone as possible. This may reduce sensitivity slightly, but will reduce the rate at which involatile material will be deposited on the sample cone.

Trifluoroacetic acid (TFA) and triethylamine (TEA) may be used up to a level of 0.05%. If solvents of high aqueous content are to be used then tuning conditions should be appropriate for the solvent composition entering the source.

Higher source temperatures (150°C) are also recommended for high aqueous content solvents. Tetrahydrofuran (THF) should ***not*** be used with peek tubing.

LC-MS Sensitivity Enhancement

The sensitivity of a LC-MS analysis can be increased or optimised in a number of ways, by alterations to both the LC operation and the MS operation.

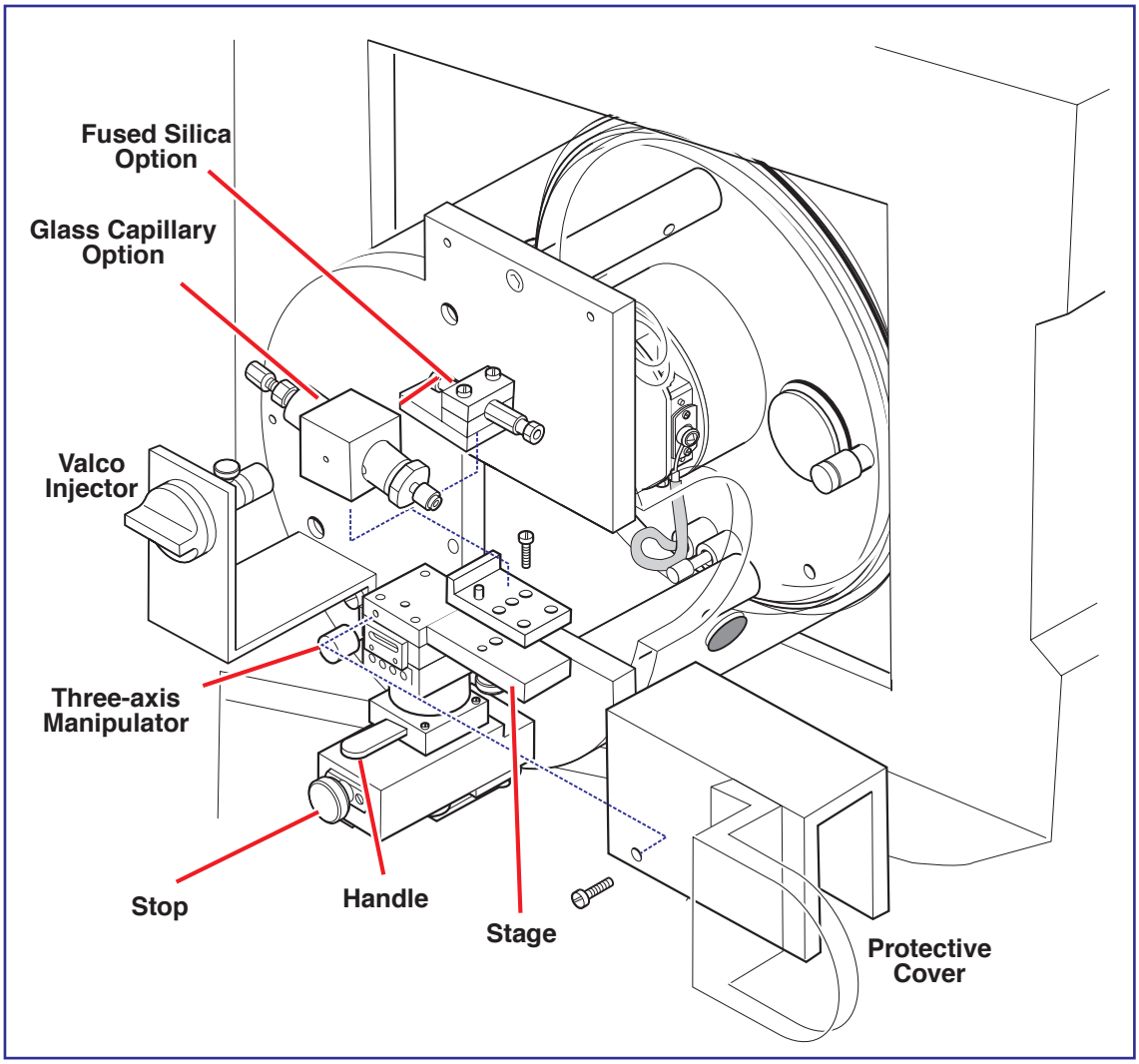
In the LC area some examples include the use of high resolution columns and columns with fully end capped packings. For target compound analysis, techniques such as trace enrichment, coupled column chromatography, or phase system switching can have enormous benefits.

Similarly, the mass spectrometer sensitivity can often be significantly increased, for instance by narrow mass scanning or by single ion recording techniques.

Careful choice of the solvent, and solvent additives or modifiers may also prove important.

Nanoflow Electrospray

Overview



The optional nanoflow interface allows electrospray ionisation to be performed in the flow rate range 5 to 1000 nanolitres per minute. There are two options for the spraying capillary, which can be alternately fitted to the interface:

- Borosilicate metal coated glass capillary.

  Metal coated glass capillaries allow the lowest flow rates to be obtained although they are used for one sample only and then must be discarded.

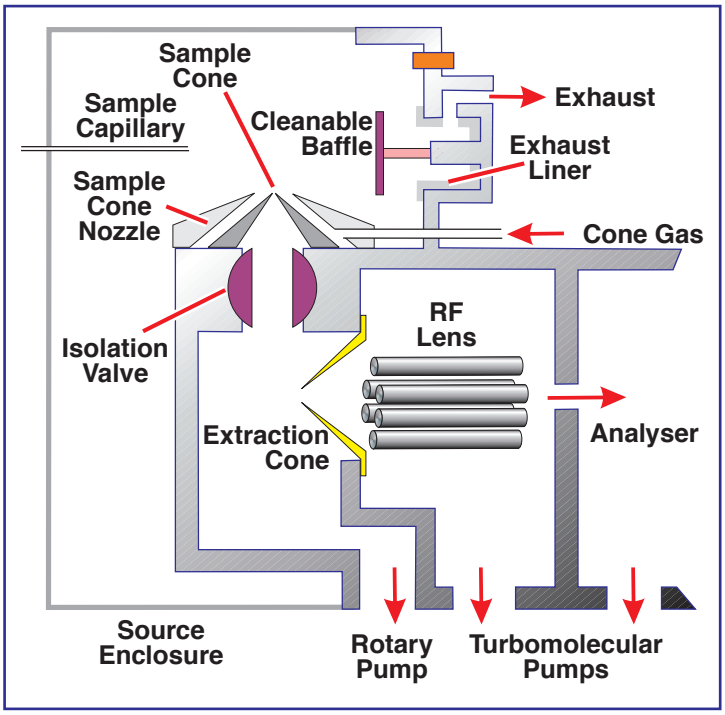
- Fused silica capillary.

  This option is suitable for flow injection analyses or for coupling to nano-HPLC, and uses a pump to regulate the flow rate down to 100 nl/min. If a syringe pump is to be used, a gas-tight syringe is necessary to obtain correct flow rates without leakage. A volume of 25µl is recommended.

For a given sample concentration, the ion currents observed in nanoflow are comparable to those seen in normal flow rate electrospray. Great sensitivity gains are therefore observed when similar scan parameters are used, due to the great reductions in sample consumption.

The nanoflow end flange consists of a three-axis manipulator, a stage, a protective cover and a stop / handle arrangement for rotation of the manipulator and stage.

The manipulator and stage are rotated by 90 degrees to change option or, in the glass capillary option, to load a new nanovial.



Caution: Failure to use the stop and handle to rotate the stage can result in permanent damage to the three-axis manipulator.

Installing the Interface

To change from the normal electrospray interface and install the nanoflow interface:

If fitted, remove the probe.

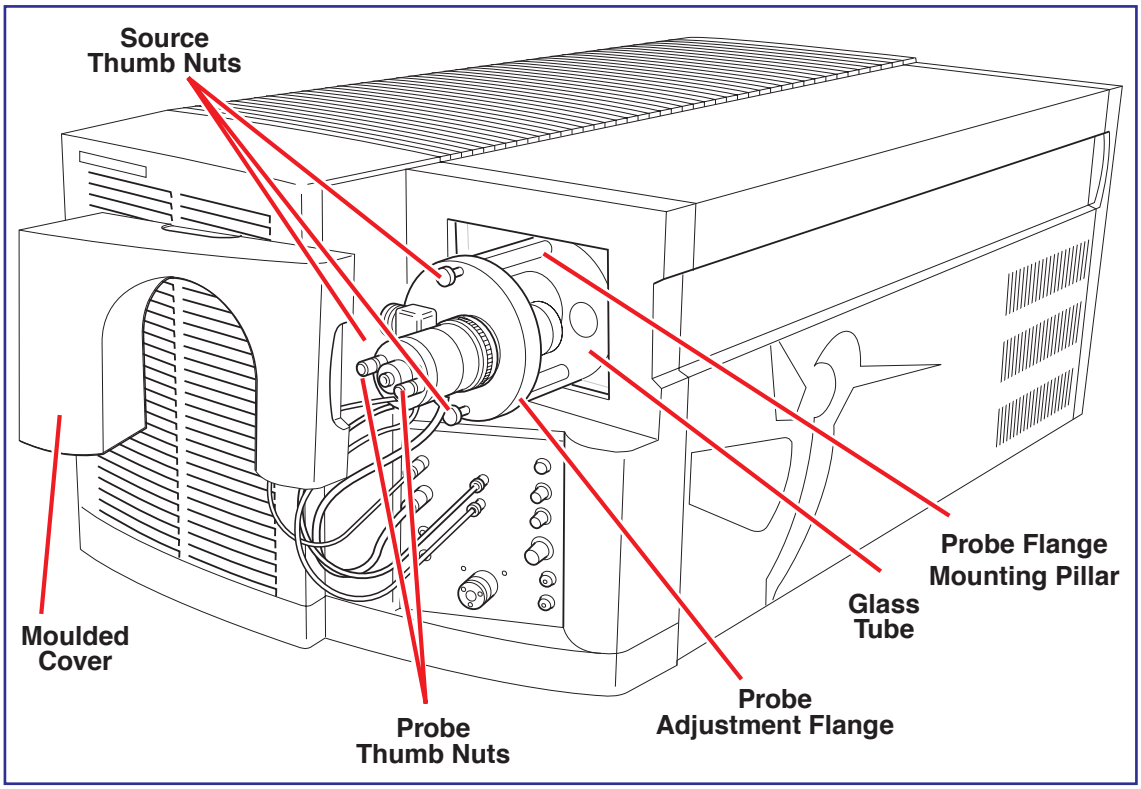
Remove the moulded cover from around the source.

Undo the three thumb screws and withdraw the probe adjustment flange assembly and glass tube.

Place the glass tube, end on, on a flat surface and place the probe support flange assembly on top of the glass tube.

Remove the PTFE encapsulated source O ring.

Warning: When the source enclosure has been removed the ion block heater is exposed. Ensure that the source block heater has been switched off and has cooled before proceeding. Observe the **Source Block Temp** readback on the tune page.



Unscrew the three probe flange mounting pillars, using the holes to obtain the necessary leverage.

If the sample cone nozzle is not in place, remove the two screws that secure the sample cone and fit the cone gas nozzle.

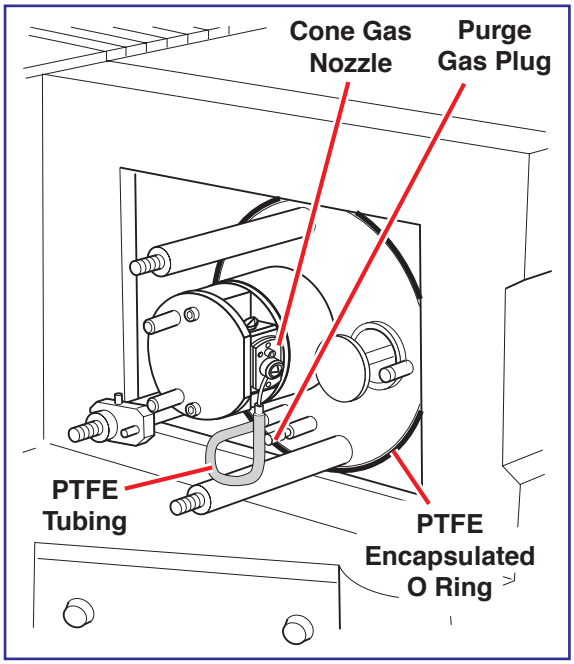
Replace the two screws.

Connect the cone gas outlet to the cone nozzle using the PTFE tubing provided.

The cone gas flow rate is set at 30% of the total desolvation gas flow.

Ensure that the purge gas is plugged (disabled).

Ensure that the cleanable baffle, the exhaust liner and the discharge pin blanking plug are fitted.



Fit a viton O ring and the three shorter nanoflow pillars.

Install the perspex cover and the nanoflow end flange, securing this with socket head screws.

Do not attempt to refit the moulded cover.

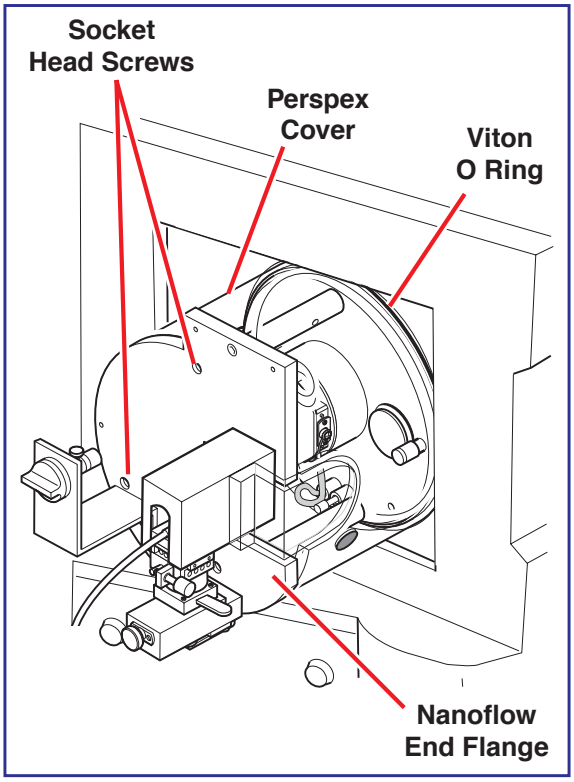
If not already in place, attach the microscope or camera brackets using the screw hole and dowels at the top of the bracket.

Insert the flexible light guide into the grommet at the base of the perspex cover.

Set the light source to its brightest.

Block the **Nebuliser** and **Desolvation Gas** outlets on the instrument's front panel.

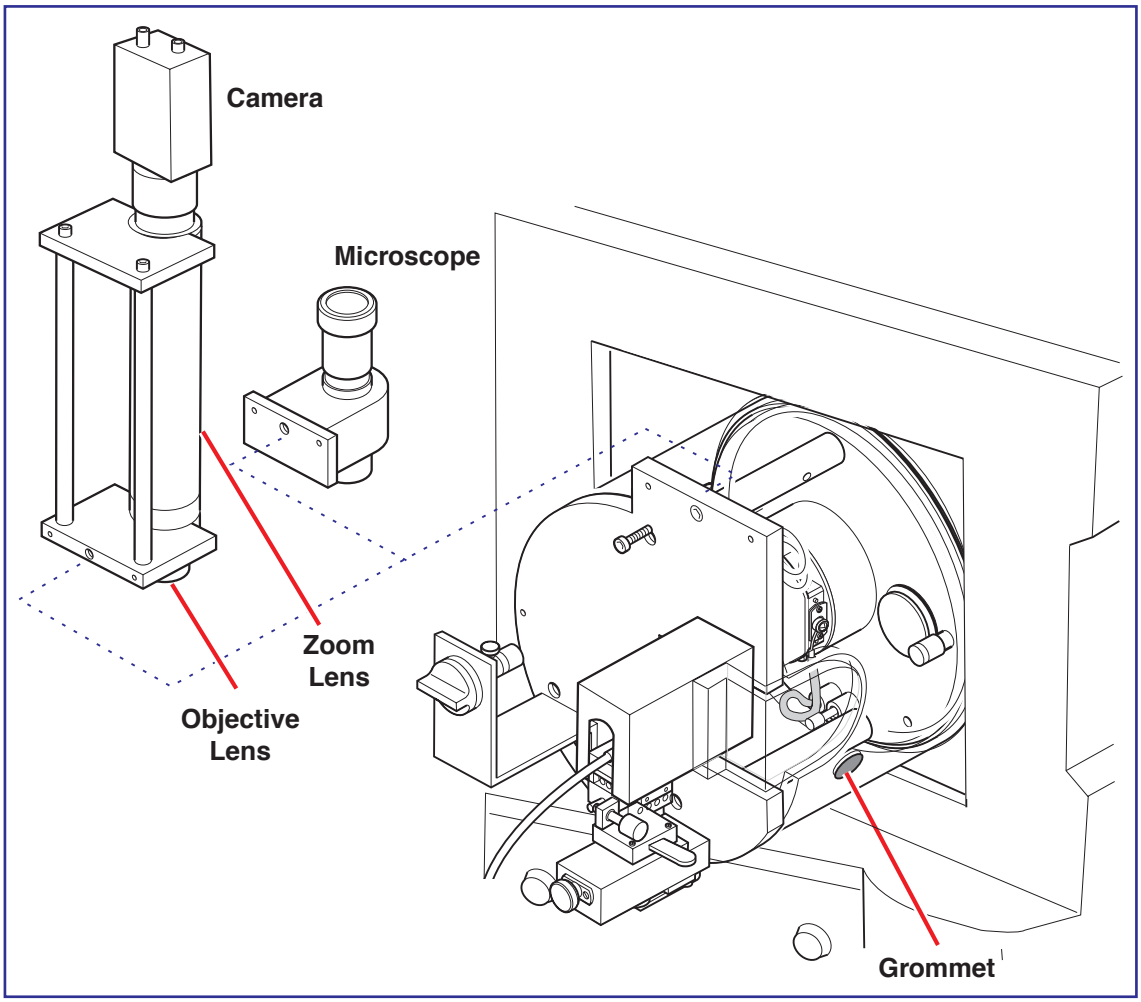
The cone gas is split from the desolvation gas internally.



Attach the two cables to the sockets marked **Capillary / Corona** and **ESI / APcI** on the front panel of the instrument.

Set **Source Block Temp** to approximately 80°C.

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.



Operation of the Camera System

Magnification is controlled by the zoom lens. A fine focus can be achieved by rotating the objective lens.

Using the Microscope

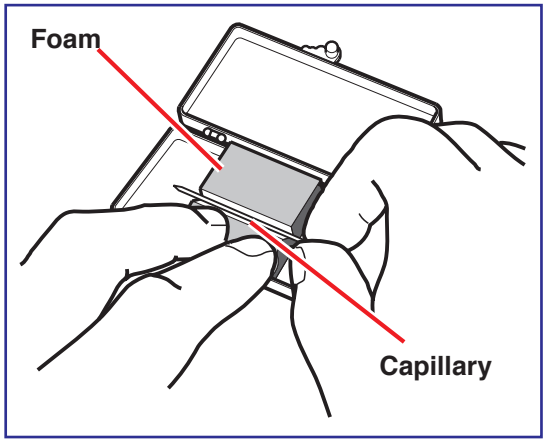
Focusing is adjusted by rotating the top of the microscope.

Glass Capillary Option

Warning: Do not touch the sharp end of the capillary. As well as the risk of injury by a sliver of glass, the needle will become inoperable.

Caution: The capillaries are extremely fragile and must be handled with great care. Always handle using the square end of the capillary.

With the stage rotated outwards, unscrew the union from the end of the assembly.



Carefully remove the capillary from its case by lifting vertically while pressing down on the foam with two fingers.

Over the blunt end of the capillary, pass the knurled nut, approximately 5mm of conductive elastomer and finally the union.

Tighten the nut (finger tight is sufficient) so that 5mm of glass capillary is protruding from the end of it. This distance is measured from the end of the nut to the shoulder of the glass capillary.

Load sample into the capillary using either a fused silica syringe needle or a gel loader tip.

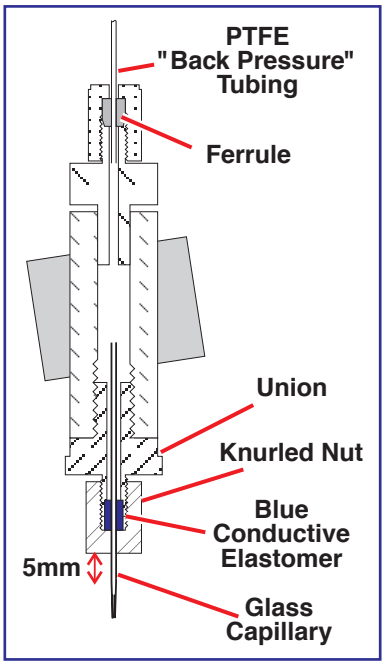
Screw the holder back into the assembly - finger tight is sufficient.

Ensure that **Capillary** is set to 0V on the tune page.

Rotate the stage back into the interface using the stop and handle.

Manoeuvre the stage so that the microscope or camera can view the capillary tip.

Using a 10ml plastic syringe or a regulated gas supply, apply pressure to the back of the tip until a drop of liquid is seen. Remove the back pressure.



On the tune page, select **Gas** and turn on **Nitrogen**.

Select OPERATE.

Set **Capillary** between 1 and 1.5kV.

Adjust **Desolvation Gas** using the knob on the front panel of the instrument.

An ion beam should now be visible on the tune page.

Tune the source voltages, adjust the gas flow and adjust the three-axis manipulator for maximum ion current.

The ion current may change dramatically with very slight changes of position but the high resolution of the threads in the manipulator allows very fine tuning.

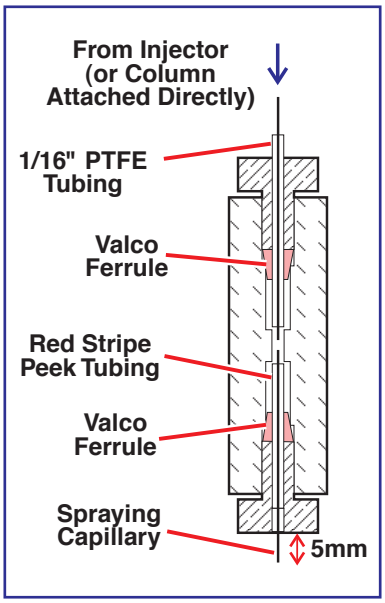
Restarting the Spray

Should the spray stop, it is possible to restart it by adjusting the three-axis manipulator so that, viewed under magnification, the capillary tip touches the sample cone and a small piece of the glass hair shears off.

It may also be necessary to apply some back pressure to the holder to force a drop of liquid from the capillary. Up to 1.4 bar (20 psi) can be applied and, with this pressure, a drop should be visible unless the capillary is blocked.

Fused Silica Option

Use the plug cap prior to assembly in the low dead volume union to set the 'pilot depth' of the ferrule on the tubing.



Plumb the low dead volume union as shown, making sure that each of the fused silica pieces are held firmly by the appropriate sleeving and a Valco ferrule.

On one side of the union is 375µm o.d., 25µm i.d. fused silica from the column or injector, sleeved to $^{1}/_{16}$ inch with PTFE tubing. On the other is the spraying capillary, 90µm o.d., 20µm i.d. fused silica, sleeved to $^{1}/_{16}$ inch with red stripe peek tubing.

Approximately 5mm of 90µm o.d. fused silica should protrude from the end of the nut as shown. This may be cut to size at the end of the assembly process.

Attach the union to the contact plate using the V-shaped clamps. Attach the plate to the manipulator stage.

Replace the protective cover and swing the stage into the interface using the stop and handle.

The Valco injector is attached to the flange by a bracket and can be used for loop injections. The injection valve is plumbed as follows:

- P from the pump.
- C to the column (or to the union).
- S is the sample port, attach a VISF sleeve here.
- W is a waste port.

Raise the flow to 1 µl/min. After a few minutes, reduce it to the desired flow rate.

To avoid a very unstable beam, it is imperative to remove any air bubbles from the fused silica lines.

Select **Gas** and switch on **Nitrogen**. Select OPERATE.

Set **Capillary** to approximately 3kV.

An ion beam should be visible.

Tune the source parameters for maximum signal.

Optimise the position of the needle using the three-axis manipulator.

In the fused silica option the needle tends to optimise further away from the cone than in the borosilicate option.

The spray from the needle should be easily visible by the microscope or camera and the stability of the spray can be seen using either system.

A column can be attached directly to the back of the union to reduce any dead volumes to a minimum, but care must be taken to match the external diameter of the column with the internal diameter of any $^{1}/_{16}$ inch sleeve.

Changing Options

To change between the glass capillary and the fused silica options:

Rotate the stage outwards.

Caution: Failure to use the stop and handle to rotate the stage can result in permanent damage to the three-axis manipulator.

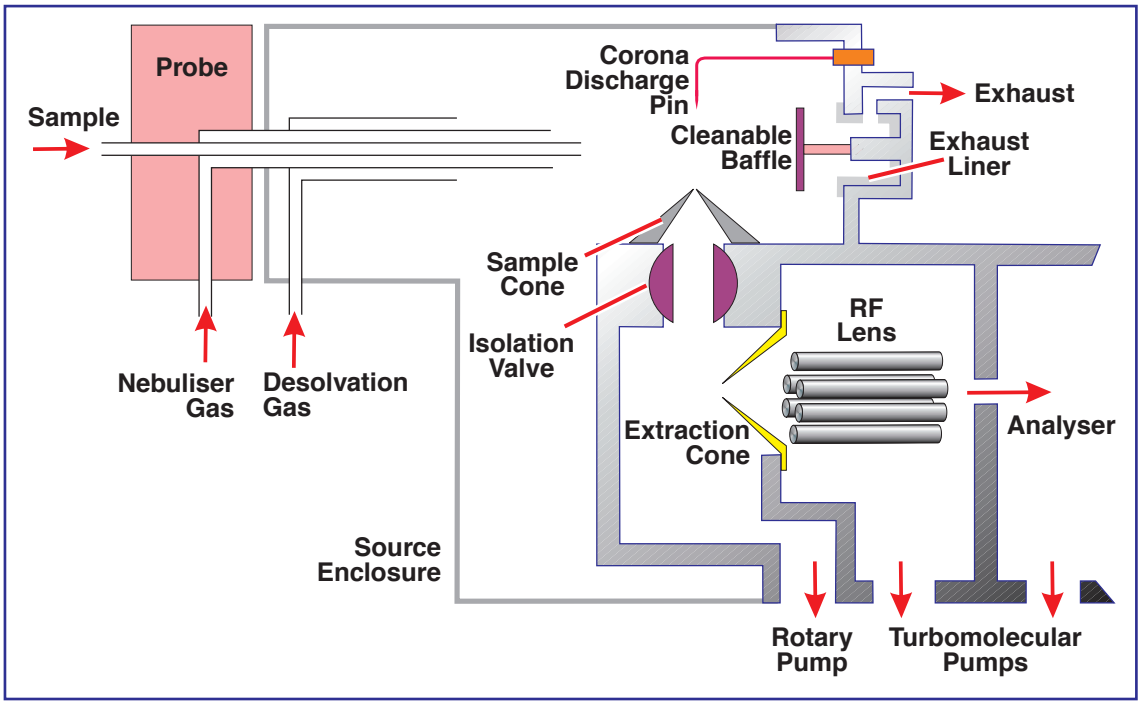
Remove the protective cover and release the captive screw located underneath the stage.

Lift off the holder and replace it with the alternative holder, securing it with the captive screw

Replace the protective cover, ensuring that either the PTFE back pressure tubing (glass capillary option) or the fused silica transfer line is fed through the slot in the back of the protective cover along with the HV cabling.

Atmospheric Pressure Chemical Ionisation

Introduction



Atmospheric Pressure Chemical Ionisation (APcI) is an easy to use LC-MS interface that produces singly-charged protonated or deprotonated molecules for a broad range of involatile analytes.

The ability to operate with 100% organic or 100% aqueous mobile phases at flow rates up to 2 ml/min makes APcI an ideal technique for standard analytical column (4.6mm i.d.) normal phase and reverse phase LC-MS.

The APcI interface consists of the standard Z-spray source fitted with a corona discharge pin and a heated nebuliser probe. Mobile phase from the LC column enters the probe where it is pneumatically converted into an aerosol and is rapidly heated and converted to a vapour / gas at the probe tip. Hot gas from the probe passes between the sample cone and the corona discharge pin, which is typically maintained at 2.5kV. Mobile phase molecules rapidly react with ions generated by the corona discharge to produce stable reagents ions. Analyte molecules introduced into the mobile phase react with the reagent ions at atmospheric pressure and typically become protonated (in positive ion mode) or deprotonated (in the negative ion mode). The sample and reagent ions pass through the sample cone into the ion block prior to being extracted into the hexapole transfer lens through the extraction cone.

Changeover between electrospray and APcI operation is simply accomplished by changing the probe and installing the corona discharge pin within the source enclosure.

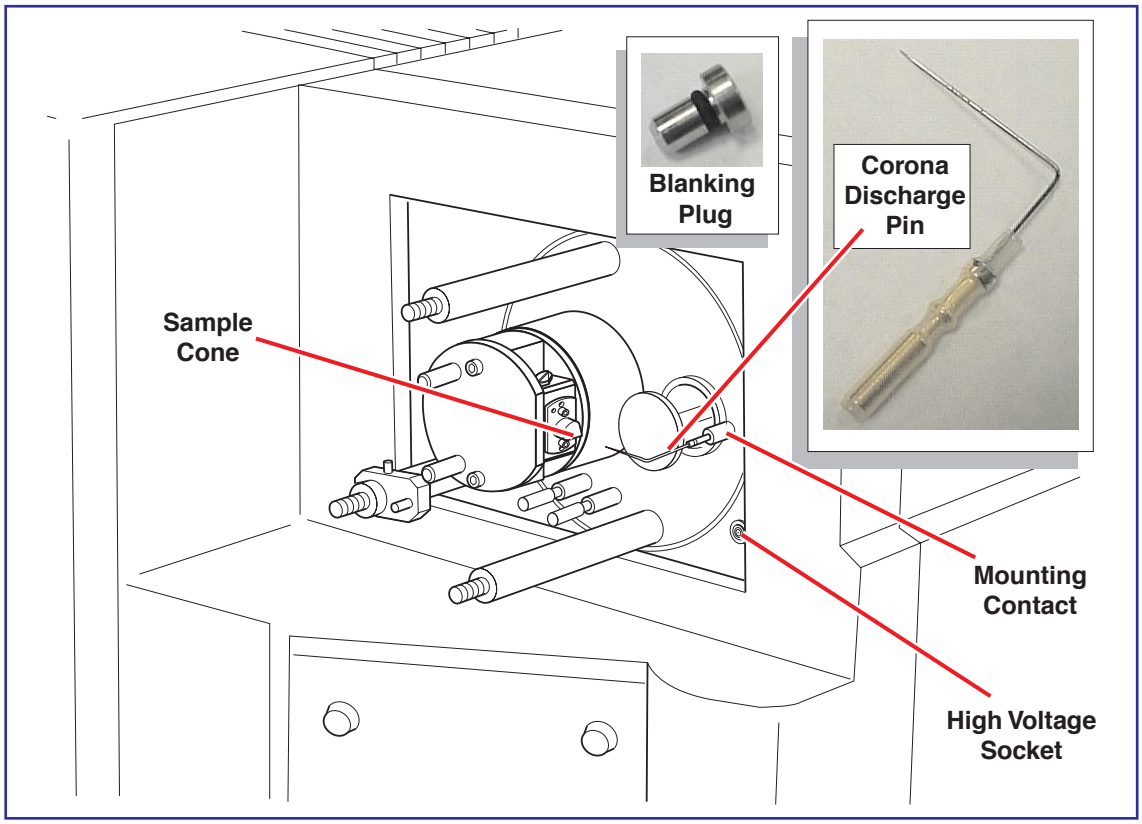
For APcI operation, the desolvation gas is not heated in the desolvation nozzle. However, it is important that desolvation gas is used throughout.

The background spectrum for 50:50 acetonitrile:water is dependent upon the settings of **Cone** and **Extractor**. The main reagent ions for typical sample and extraction voltages of 40V and 3V respectively are 42, 56, 83 and 101.

Acetonitrile adducting may be minimised by optimisation of the probe position.

Preparation

Ensure that the source is assembled as described in *Maintenance and Fault Finding*, and that the instrument is pumped down and prepared for APcI operation as described in *Routine Procedures*.



APcI may be operated with or without the cleanable baffle fitted.

Ensure that a supply of nitrogen has been connected to the gas inlet at the rear of the instrument and that the head pressure is between 6 and 7 bar (90-100 psi).

Checking the Probe

Ensure that the probe heater is off.

Unplug the probe from the instrument's front panel and remove the probe from the source.

Connect the PTFE tube to the **Nebuliser** outlet on the front panel.

Remove the probe tip assembly by carefully loosening the two grub screws.

Disconnect the heater from the probe body by pulling parallel to the axis of the probe.

Ensure that 0.5 to 1mm of fused silica is protruding from the stainless steel nebuliser tube.

Connect the LC pump to the probe with a flow of 50:50 acetonitrile:water at 1 ml/min.

Check that the liquid jet flows freely from the end of the capillary and that the LC pump back pressure reads 250 to 400 psi.

Check that the nitrogen supply pressure is 6 to 7 bar (90 to 100 psi).

Select **Gas** and turn on **Nitrogen**.

Check that the liquid jet converts to a fine uniform aerosol.

Switch off the liquid flow.

Select **Gas** and turn off **Nitrogen**.

Reconnect the probe tip assembly.

Insert the APcI probe into the source and secure it by tightening the two thumb screws.

Connect the probe cable to **APcI / ESI** on the instrument's front panel.

The plug labelled **ESI** *must first be unplugged from the front panel.*

Obtaining a Beam

Ensure that the corona discharge pin is fitted as described in *Routine Procedures, Preparation for APcI Operation* and that the pin is connected using the APcI HV cable.

Ensure that the APcI probe is fitted as described above, that the desolvation gas tube is connected to the front panel, and that the cone gas and purge gas outlets are plugged.

The top line of the tune page indicates the current ionisation mode.

If necessary, change the ionisation mode using the **Ion Mode** command.

Set **Source Block Temp** to 150°C.

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Set **APcI Probe Temp** to 20°C with no liquid flow and **Nitrogen** off.

Initially set **Corona** to 2.5 kV and **Cone** to 30V.

When **Source Block Temp** reaches 150°C:

Select **Nitrogen** to switch on the nitrogen gas.

Using the valves on the front of the instrument, adjust **Desolvation Gas** to 150 litres/hour and set **Nebuliser Gas** to its maximum setting.

To monitor the flow rate, select **Window** *then* **Gas Flow** *on the tune page and observe the readback window.*

Select one of the peak display boxes and set **Mass** to 50 and **Span** to 90.

Select OPERATE.

Increase **Gain** on the peak display box in the range 1 to 20 until peaks become clearly visible.

Set **APcI Probe Temp** to 400°C.

When **APcI Probe Temp** reaches 400°C:

Start the LC pump at a flow of 1 ml/min.

Optimise **Corona** so that the peaks reach maximum intensity.

Optimise the probe position for intensity and stability.

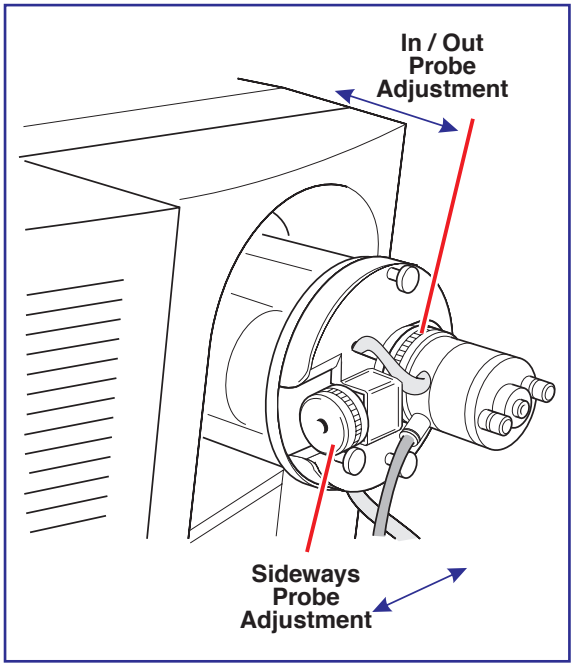
The two screws can be adjusted singly or simultaneously to optimise the beam.

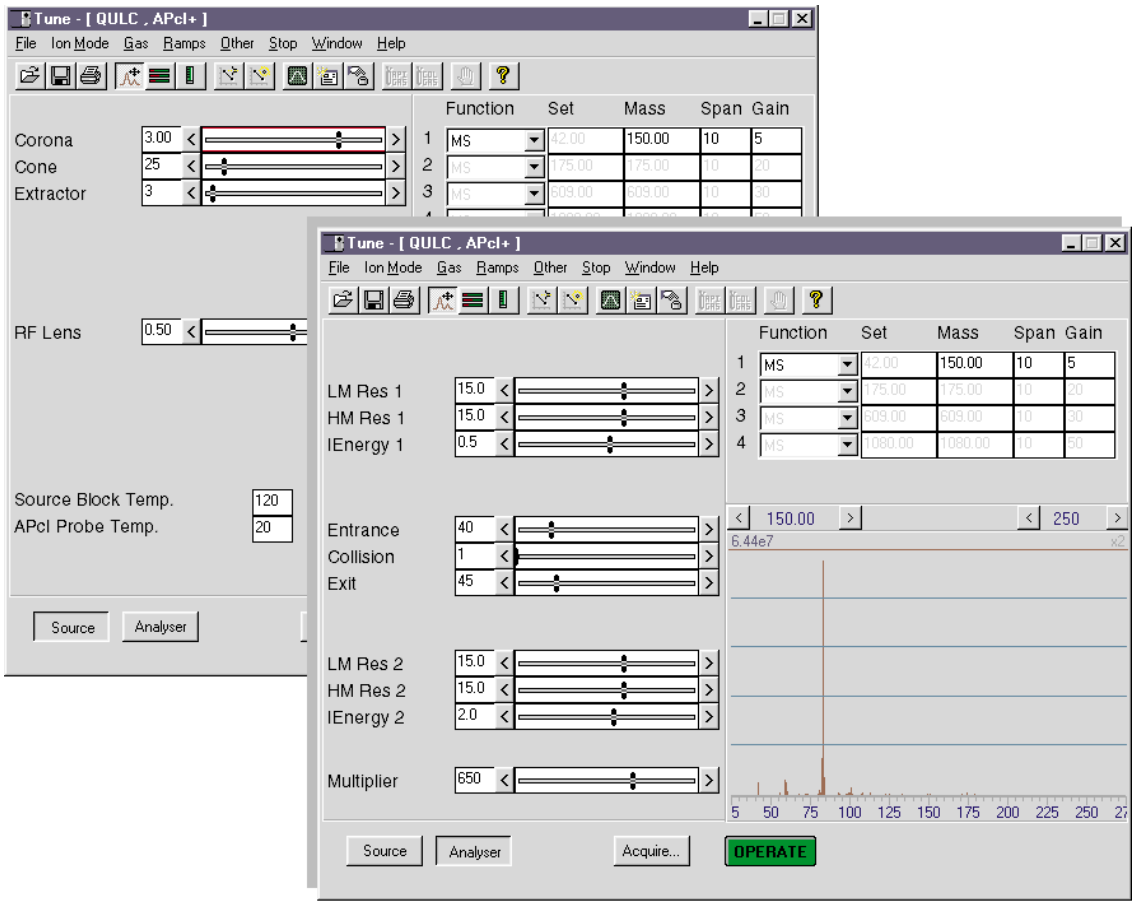
The position of the probe will affect sensitivity. However, if the sample is contained in a 'biological matrix' or is contained in an involatile solvent the probe should be moved away from the sample cone and towards the corona discharge pin.

Warning: It is normal for the source enclosure, the glass tube and parts of the probe adjustment flange to reach temperatures of up to 60°C during prolonged APcI operation. Care should be exercised when handling source components immediately after operation.

Warning: Switch off the liquid flow and allow the probe to cool (<100°C) before removing it from the source.

Caution: Failure to employ a desolvation gas flow during APcI operation may lead to heat damage to the source.





Calibration

Having obtained a stable APcI beam, refer to *Mass Calibration* later in this document.

Hints for Sample Analysis

Tuning

- Start by tuning on the solvent ions.
- It is generally found that the most significant analyte tuning parameter to adjust following tuning on the solvent ions is **Cone**.
- Fine tuning on the analyte of interest can be performed either by large loop injections (100µl) or by constant infusion in the mobile phase typically at analyte concentrations of a few ng/µl.
- 10µl loop injections can be monitored using real time chromatogram updates.

Mobile Phase

- The choice of mobile phase is an important compound specific factor in APcI. For example, steroids prefer methanol:water mixtures as opposed to acetonitrile:water.
- Analyte sensitivity is also dependent on mobile phase composition, which can be varied from 100% aqueous to 100% organic for any particular mixture.

Probe Temperature

This can be a critical factor for some analytes.

- Involatile samples (for example steroids) generally require high probe temperatures (>400°C).
- Volatile samples (for example pesticides) can be analysed with low probe temperatures (<400°C).
- In some cases, too high a probe temperature can lead to thermal degradation of labile samples.

Desolvation Gas

Although a **Desolvation Gas** flow of approximately 150 litres/hour is typical for most samples, this flow rate should be tuned for maximum sensitivity while ensuring that the flow rate is not decreased below 100 litres/hour.

Removing the Probe

After a session of APcI operation:

Turn off the LC flow.

Set **APcI Probe Temp** to 20°C**.**

Deselect OPERATE.

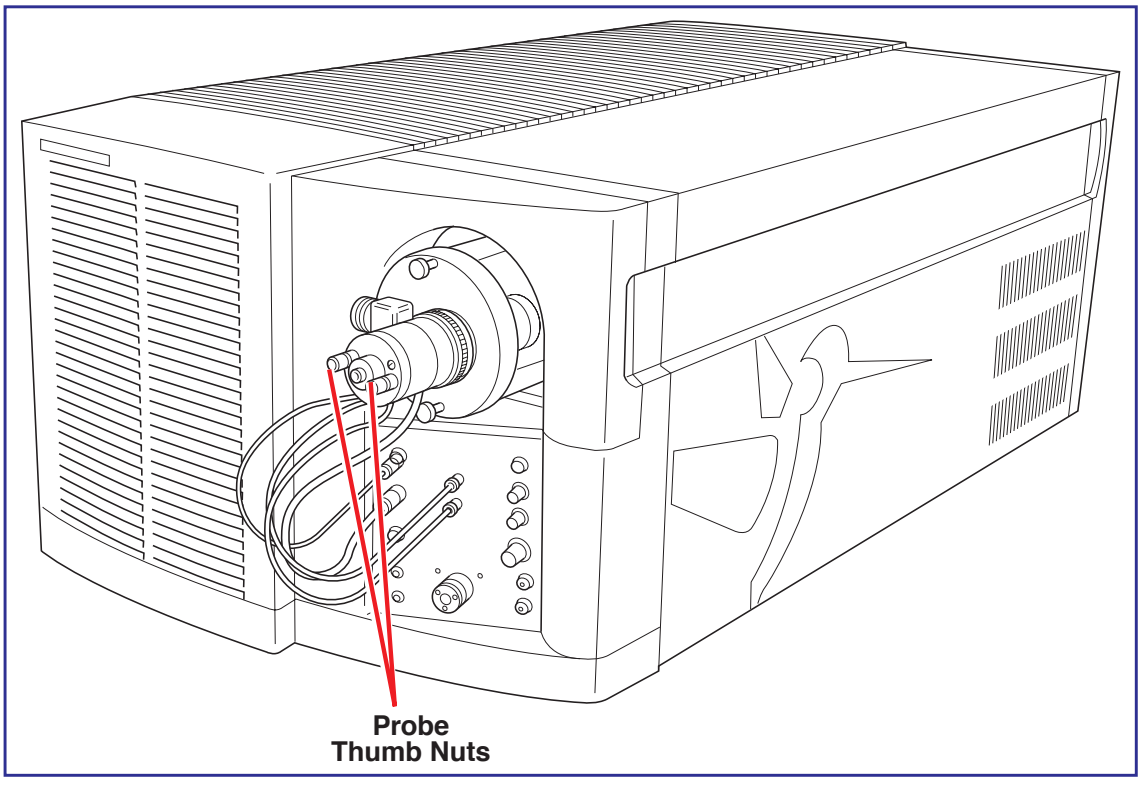
When the probe temperature falls below 100°C:

Select **Gas** and turn off **Nitrogen**.

Undo the two thumb nuts and remove the probe from the source.

Warning: Take care when removing the APcI probe. There is a risk of burns to the operator.

Caution: Removal of the APcI probe when hot will shorten the life of the probe heater.



If the instrument is not to be used for a long period of time the source temperature should be reduced to 60°C.

Mass Calibration

Introduction

MassLynx NT allows a fully automated mass calibration to be performed, which covers the instrument for static and scanning modes of acquisition over a variety of mass ranges and scanning speeds.

The first section of this chapter describes a complete mass calibration of Quattro LC using electrospray ionisation with a mixture of sodium iodide and rubidium iodide as the reference compound. The second section describes a similar procedure using atmospheric pressure chemical ionisation (APcI) with PEG as the reference compound.

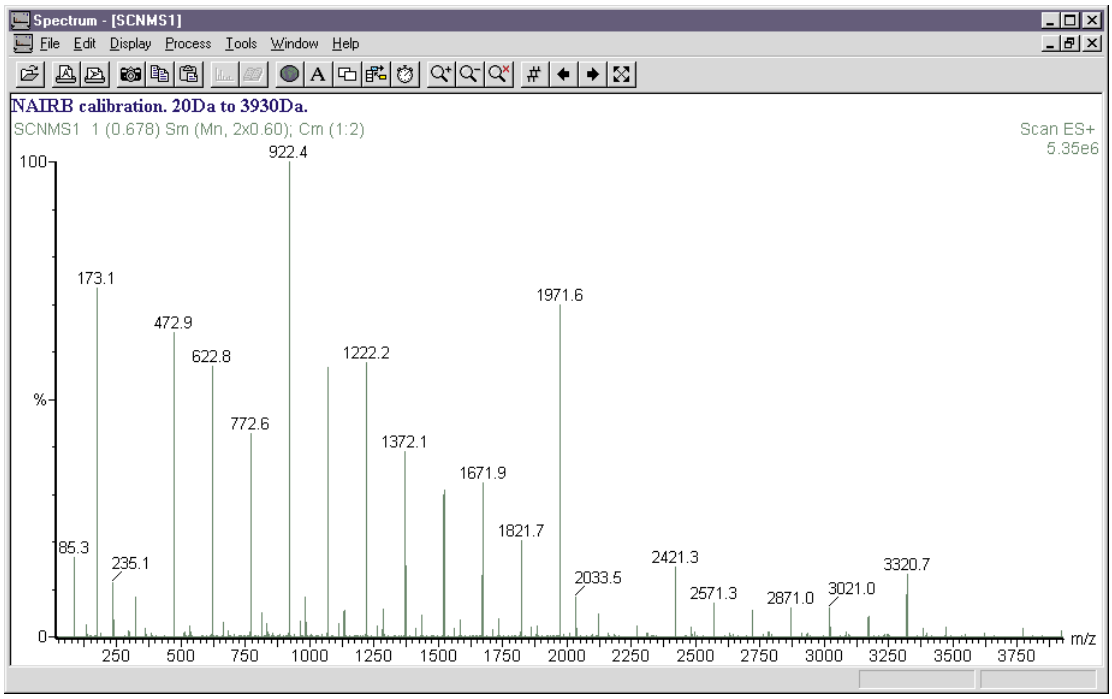
See *Reference Information* for details of calibration solutions and their preparation.

Electrospray

Overview

When a calibration is completed it is possible to acquire data over any mass range within the calibrated range. It is therefore sensible to calibrate over a wide mass range.

With a mixture of sodium iodide and rubidium iodide calibration over the instrument's full mass range is achievable.



Preparing for Calibration

Reference Compound Introduction

The example given here describes an automatic calibration which requires reference compound to be present for several minutes. The introduction of the reference compound is best achieved using a large volume Rheodyne injector loop (50 or 100µl) or an infusion pump (for example, a Hamilton syringe pump).

When using a large volume injection loop:

Set up a solvent delivery system to deliver 4-5 µl/min of 50:50 acetonitrile:water or 50:50 methanol:water through the injector into the source.

An injection of 50µl of reference solution will then last for at least 10 minutes.

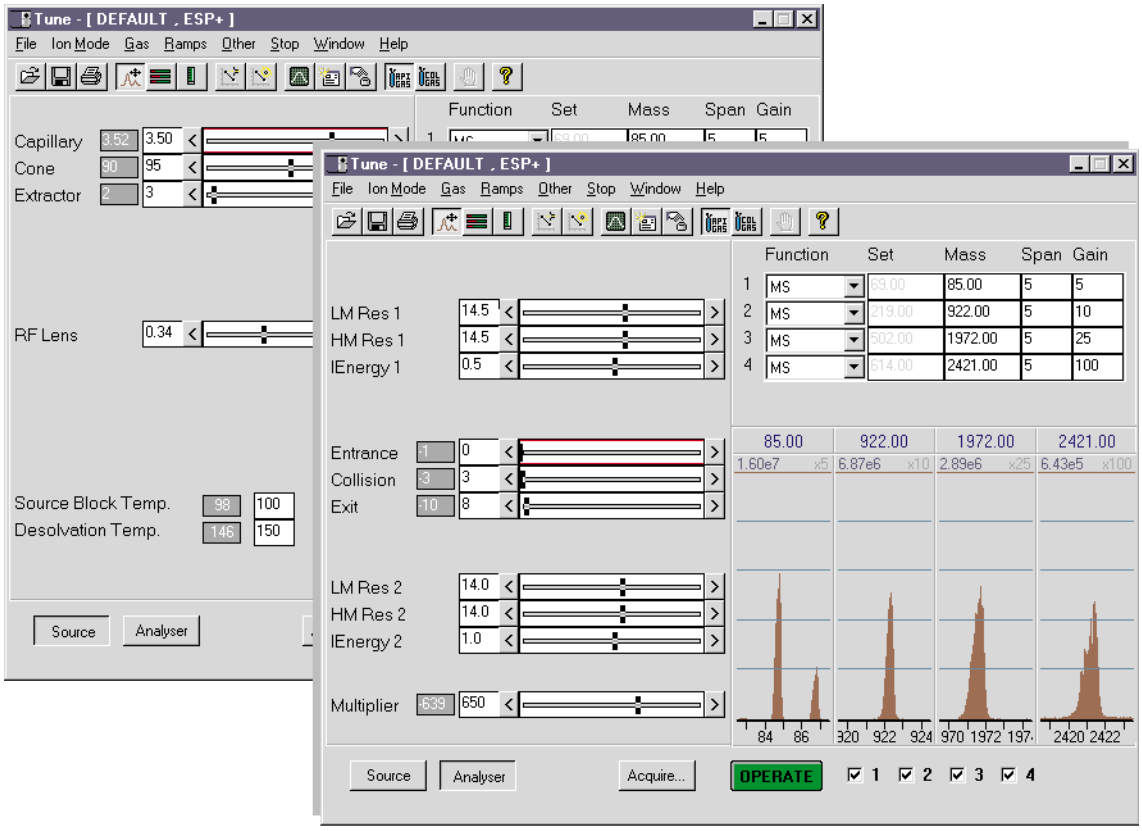
When using an infusion pump:

Fill the syringe with the reference solution.

Couple the syringe to the electrospray probe with fused silica tubing.

Set the pump to a flow rate of 4-5 µl/min.

Tuning



Before beginning calibration, and with reference solution admitted into the source:

Set **Multiplier** to 650V.

Adjust source parameters to optimise peak intensity and shape.

Set the resolution and ion energy parameters for unit mass resolution on MS1 and MS2.

For a good peak distribution across the full mass range:

Check the intensity of some of the reference peaks above 1000 amu.

Check also the intensity of the peaks at m/z 85 and 173.

A cone voltage in the region of 90 is usually suitable.

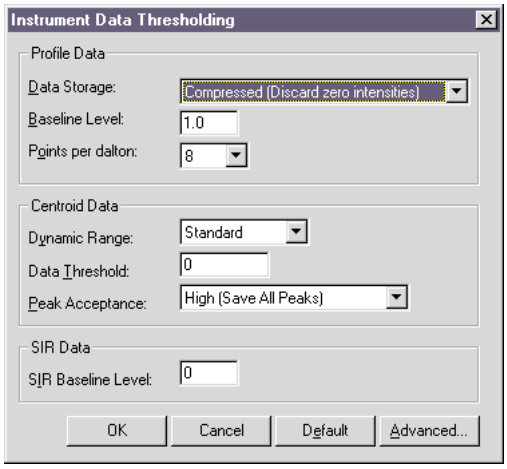
The peak at 23 is Na, the 85 peak is rubidium and the others are NaI clusters.

A typical tune page is shown above.

Instrument Threshold Parameters

Before beginning the calibration procedure, some instrument parameters need to be checked. For most low mass range calibrations, calibration data is acquired in continuum mode. To allow suitable scanning speeds to be used the continuum data parameters need to be set correctly.

From the instrument control panel select **Instrument** then **Set Instrument Threshold** to display the Instrument Data Thresholding window.

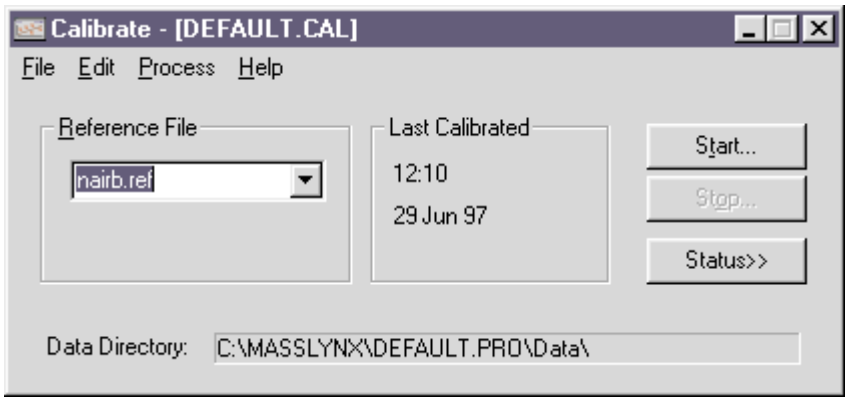


In the **Profile Data** section select **Compressed (Discard zero intensities)** for Data **Storage and** 8 **Points per Dalton.**

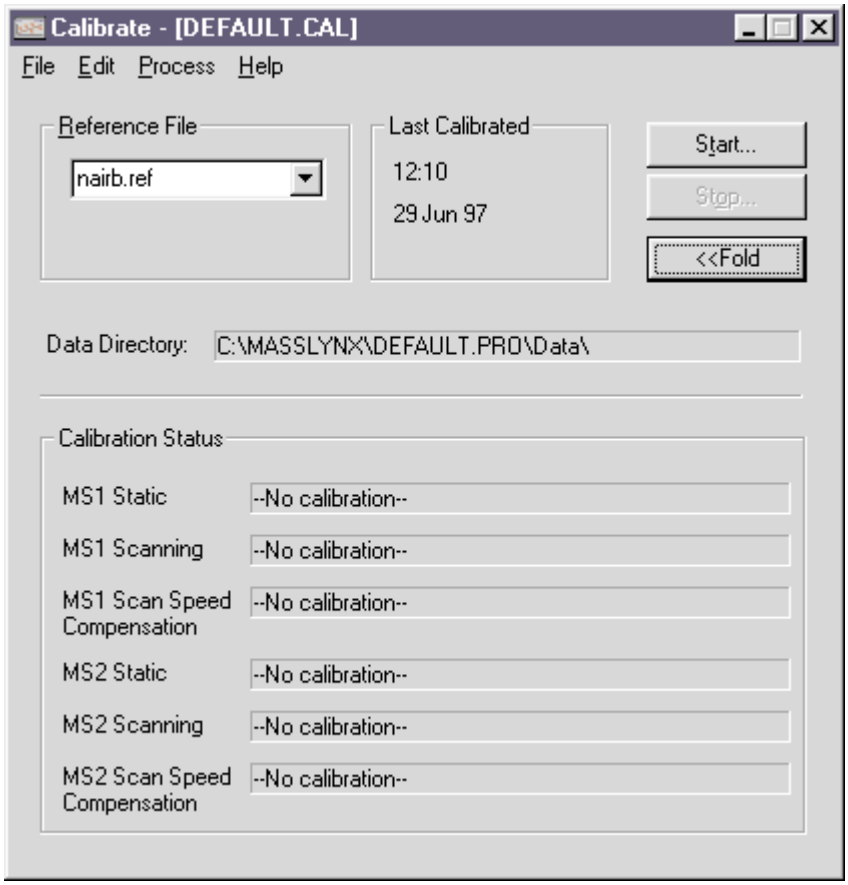
Select OK to save the parameters.

Calibration Options

To access the calibration options click on **Instrument** then **Calibrate** on the acquisition control panel. This brings forward the calibration dialog box which can be displayed in two forms. The abbreviated form, shown below, shows the selected reference file and the date the instrument was last calibrated.



Selecting the **Status>>** button displays the full dialog box which shows the current calibration status. Selecting the **<<Fold** button returns to the abbreviated box.



Selecting the Reference File

Click on the arrow in the **Reference File** box and scroll through the files until the appropriate file can be selected.

Select **nairb.ref** for a sodium iodide and rubidium iodide reference solution covering the range 23 to 3920 amu.

Removing Current Calibrations

Select the **uncal.cal** calibration file from the **File**, **Load Calibration...** menu option.

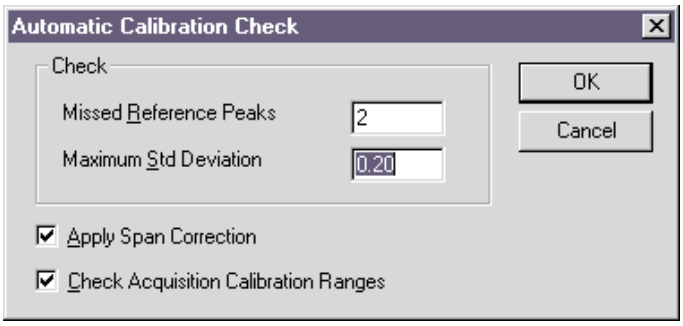
Select **Process**, **Delete all calibration...** followed by **File**, **Save Calibration**.

This ensures that a file with no calibration is currently active on the instrument and prevents any previously saved calibrations from being modified or overwritten.

Selecting Parameters

A number of parameters needs to be set before a calibration is started. Default parameters are set when the software is initially loaded which usually give a suitable calibration, but under some conditions these may need to be adjusted.

Automatic Calibration Check



This is accessed from **Edit**, **AutoCal Check Parameters...**. It is here that limits are set which the calibration must attain before the instrument is successfully calibrated. Two user parameters can be set.

Missed Reference Peaks sets the maximum number of consecutive peaks which are not matched when comparing the reference spectrum and the acquired calibration spectrum. If this number is exceeded then the calibration will fail. The default value for this parameter, 2, is suitable in most cases.

Maximum Std Deviation is set to a default of 0.20. During calibration the difference between the measured mass in the acquired calibration file and the true mass in the reference file is taken for each pair of matched peaks. If the standard deviation of the set of mass differences exceeds the set value then the calibration will fail. Reducing the value of the standard deviation gives a more stringent limit. Increasing the standard deviation means that the requirement is easier to meet, but this may allow incorrect peak matching. Values greater than 0.20 should not be used unless exceptional conditions are found.

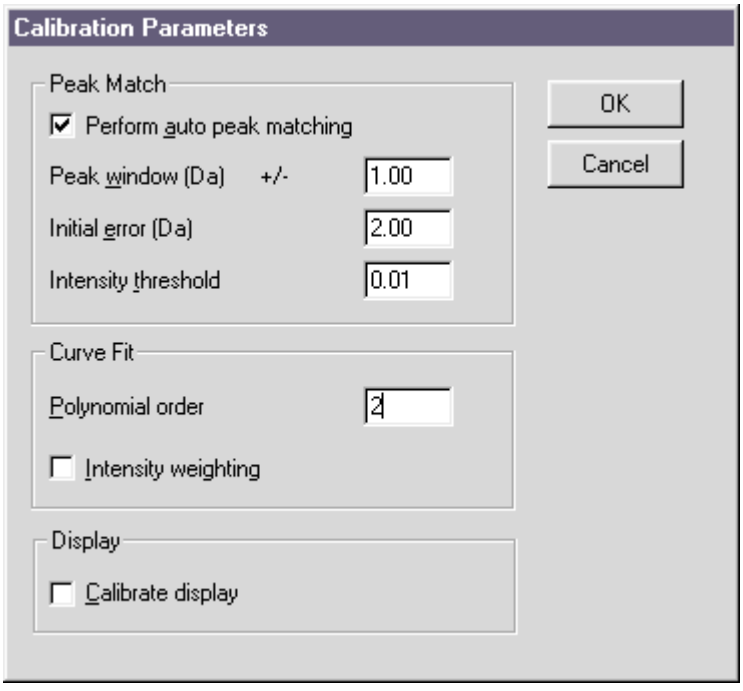
Apply Span Correction should always be left on. This allows different mass ranges to be scanned, within the calibrated range, without affecting mass assignment.

Check Acquisition Calibration Ranges causes warning messages to be displayed if an attempt is made to acquire data outside of the calibrated range for mass and scan speed. It is advisable to leave this on.

Calibration Parameters

These are accessed from **Edit, Calibration Parameters...**.

The **Peak Match** parameters determine the limits within which the acquired data must lie for the software to recognise the calibration masses and result in a successful calibration. These parameters are described in detail in the *MassLynx NT User's Guide*. The default values are shown below.



Increasing the **Peak window** and **Initial error** gives a greater chance of incorrect peak matching. All peaks in the acquired spectrum below the **Intensity threshold** value (measured as a percentage of the most intense peak in the spectrum) will not be used in the calibration procedure.

The process of producing a calibration curve is described in detail in the *MassLynx NT User's Guide*. The **Polynomial order** of the curve has values from 1 to 5 as the available options:

A polynomial order of 1 should not be used.

An order of 2 is suitable for wide mass ranges at the high end of the mass scale, and for calibrating with widely spaced reference peaks. Sodium iodide in particular has widely spaced peaks (150 amu apart), and horse heart myoglobin is used to calibrate higher up the mass scale, so this is the recommended polynomial order for these calibrations.

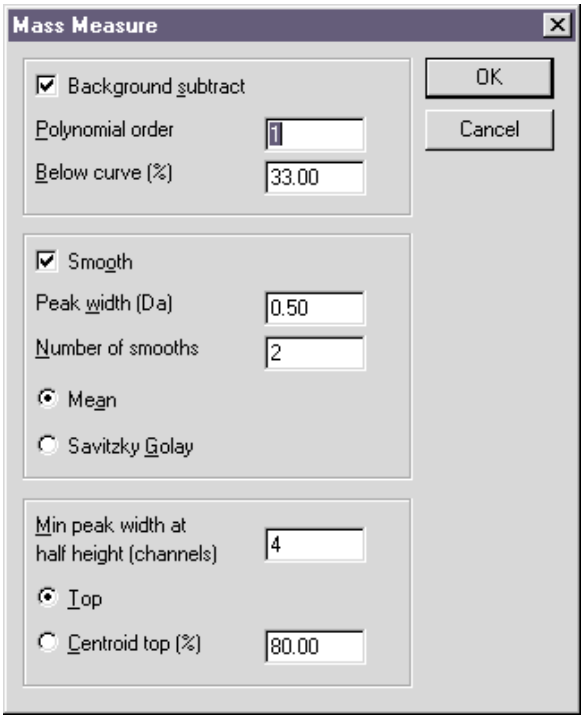
An order of 3 fits a cubic curve to the calibration.

A fourth order is used for calibrations which include the lower end of the mass scale, with closely spaced reference peaks. This is suitable for calibrations with PEG which extend below 300 amu..

A fifth order fit rarely has any benefit over a fourth order fit.

Mass Measure Parameters

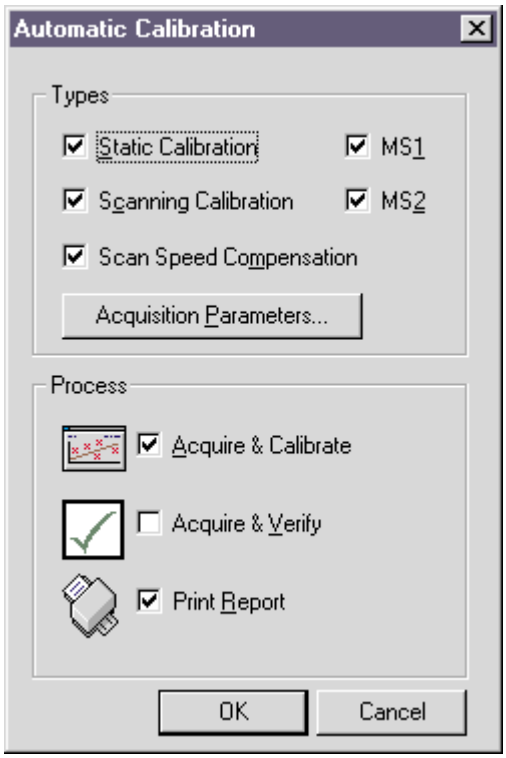
These are accessed through **Edit**, **Mass Measure Parameters...**. If continuum or MCA data are acquired for calibration then these parameters need to be set before the calibration is carried out. Information on these parameters can be found in the *MassLynx NT User's Guide*. If centroided data are used for calibration then the mass measure parameters are not used.



With electrospray calibrations, particularly with sodium iodide which has some low intensity peaks at higher mass, it is recommended that continuum or MCA data are acquired.

Performing a Calibration

Three types of calibration are available with MassLynx: static calibration, scanning calibration and scan speed compensation. These are selected on the Automatic Calibration dialog box (see below) which is accessed by selecting the **Start...** button from the Calibrate dialog box.



It is recommended that all three types of calibration are performed so that any mode of data acquisition can be used and mass ranges and scan speeds can be changed whilst maintaining correct mass assignment. However, it is possible to have any combination of these calibrations:

- If only a static calibration is present then the instrument is calibrated for acquisitions where the quadrupoles are held at a single mass as in SIR or MRM.
- If only a scanning calibration is present then the instrument is only correctly calibrated for scanning acquisitions over the same mass range and at the same scan speed as those used for the calibration.
- If only a scan speed compensation is present (with no scanning calibration having been performed) then the scan speed compensation is treated as a scanning calibration and the instrument is only correctly calibrated for scanning acquisitions over the same mass range and at the same scan speed as used for the calibration.

  For the scan speed compensation to be used correctly a scanning calibration should also be performed.

- If static and scanning calibrations are both present, then the instrument is calibrated for acquisitions where the quadrupole is held at a single mass and for scanning acquisitions with a mass range which lies within the mass range of the scanning calibration providing that the same scan speed is used.

  For example, if the instrument is calibrated from $^{m}/_{z}$ 100 to 900 with a 2 second scan (400 amu/sec) then data can be acquired from 100 - 500 amu with a 1 second scan time (also 400 amu/sec) whilst maintaining correct mass assignment. In this case the static calibration would be used to determine the start mass of the acquisition and the scanning calibration would be used for mass assignment and scan range.

- If scanning calibration and scan speed compensation are present then the instrument is only calibrated for scanning acquisitions over the same mass range as that used for the calibration, but the scan speed can be changed provided that it remains within the scan speeds used for the two calibrations. The mass range should not be changed as there is no static calibration to locate the start mass.
- If all three types of calibration are present then all types of acquisition can be used providing that the mass range and scan speed are between the lower and upper limits used for the scanning calibration and the scan speed compensation.

For a complete calibration:

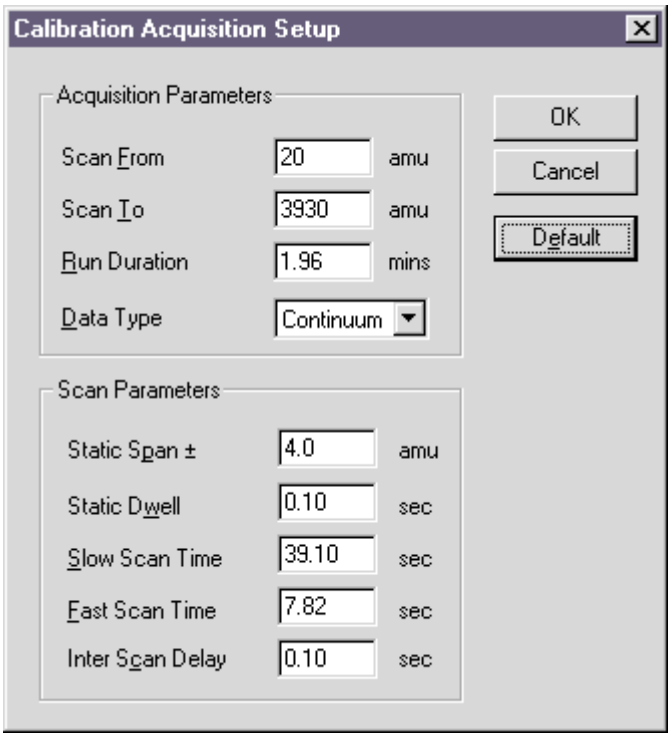
Check the boxes in the **Types** area of the dialog box adjacent to **Static Calibration**, **Scanning Calibration** and **Scan Speed Compensation**. Check also the **MS1** and **MS2** boxes.

In the **Process** area of the dialog box check **Acquire & Calibrate** and **Print Report**.

Acquisition Parameters

Selecting the **Acquisition Parameters...** button in the Automatic Calibration dialog box brings forward a second box, shown below, where the mass ranges, scan speeds and acquisition mode are set. When this box is first accessed it will contain default parameters relevant to the chosen reference file. These default parameters show the limits of scan range and scan speed for the currently selected instrument and calibration parameters.

The upper area contains the **Acquisition Parameters** where mass range, run time and data type are set.



When the instrument is fully calibrated any mass range or scan speed is allowed within the upper and lower limits dictated by the calibrations.

If the **nairb.ref** file is selected, the default button will give the parameters shown above. The solution described in *Reference Information* is suitable for use with this reference file.

If compatible reference solutions and reference files are used, then simply selecting the default button is sufficient action - no parameters need be entered manually.

Run Duration sets the time spent acquiring data for each part of the calibration. The time set must allow a minimum of three scans to be acquired at the slowest scan speed used. If the run duration is too short then data will not be acquired. The slowest scan speed generally used is 100 amu/sec. With **Scan From** set to 20 amu and **Scan To** set to 2000 amu a scan time of 19.8 seconds is required, and an **Inter Scan Delay** (in the lower area of the box) of 0.1 second is usually used. Therefore the run duration must be greater than 59.6 seconds (3 scans + 2 inter scan delays). A **Run Duration** of 1.00 minutes is suitable.

Data Type allows a choice of centroided, continuum or MCA data to be acquired. **Continuum** or **MCA** acquisitions are generally used for electrospray calibrations, although calibrating in MCA mode limits the maximum acquisition speed to 400 amu/sec. When using thresholded continuum data with 8 channels per amu (see the *MassLynx NT User's Guide*) the maximum acquisition speed is 1000 amu/sec.

The lower area in the Calibration Acquisition Setup dialog box contains the **Scan Parameters**.

When an instrument acquires data for a static calibration it examines the reference file to find the expected reference masses, and then acquires data over a small mass span around each peak's expected position. Thus the acquired data do not contain continuous scans. Each spectrum comprises small regions of acquired data around each peak, separated by regions where no data are acquired.

Static Span sets the size of this small region around each reference peak. A span of 4.0 amu is typical.

Static Dwell determines how much time is spent acquiring data across the span. A value of 0.1 second is suitable.

Slow Scan Time determines the scan speed used for the scanning calibration. If both a scanning calibration and a scan speed compensation are to be performed then the scan speed should be set to approximately 100 amu/sec (a scan time of 19.8 seconds over a mass range of 20 to 2000 amu). If only a scanning calibration is to be performed (without scan speed compensation) then the scan speed should be set at the same speed to be used for later acquisitions.

Fast Scan Time determines the scan speed used for the scan speed compensation, and the upper limit of scan speed that can be used for subsequent acquisitions. When using MCA or thresholded continuum data the scan speed is limited to 400 and 1000 amu/sec respectively. So, for a mass range of 100 to 1600 amu, the minimum values are 1.5 seconds for thresholded continuum data and 2.5 seconds for MCA data. When using centroided data the maximum acquisition rate is much higher, although it is unlikely that scan speeds of greater than 2000 amu/sec would be needed for acquiring data.

Select **Default** then **OK** to return to the Automatic Calibration dialog box. Alternatively, select chosen values if a different calibration range is required.

Starting the Calibration Process

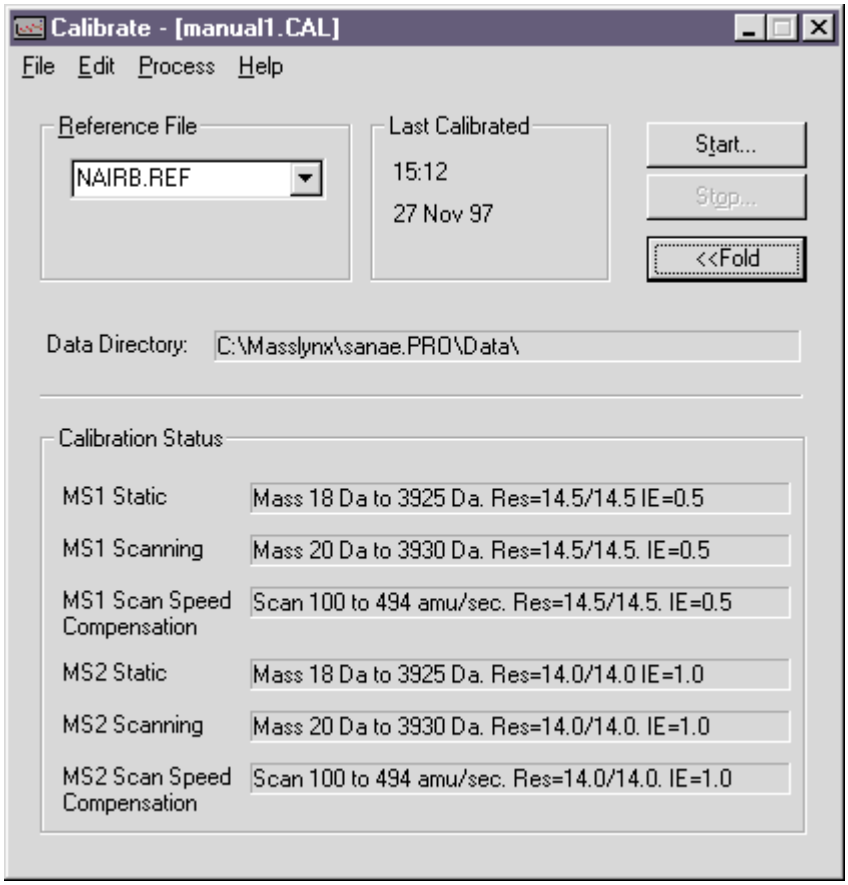
To start the calibration process:

Select **OK** from the Automatic Calibration dialog box.

The instrument acquires all of the calibration files in the following order using the data file names shown:

MS1 static calibration	data file: STATMS1
MS1 scanning calibration	data file: SCNMS1
MS1 scan speed compensation	data file: FASTMS1
MS2 static calibration	data file: STATMS2
MS2 scanning calibration	data file: SCNMS2
MS2 scan speed compensation	data file: FASTMS2

Once all of the data have been acquired each data file is combined to give a single spectrum which is then compared against the reference spectrum to form a calibration. This process takes place in the same order as above. If the full calibration dialog box is open then a constantly updated status message for the calibration is displayed.



If, when the process is completed, the calibration statistics meet with the requirements specified by the selected calibration parameters then a successful calibration message is displayed. A calibration report is then printed showing a calibration curve for each of the calibration processes. An example of a calibration report is shown opposite.

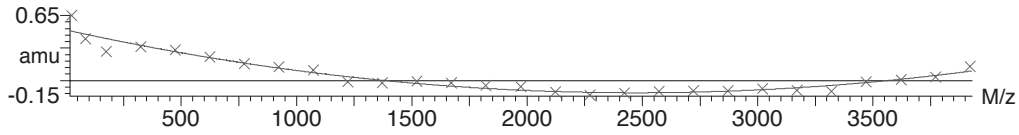
Instrument Calibration Report. Page 1

Mass 18 Da to 3925 Da. Res=14.5/14.5 IE=0.5

Calibrated - 15:12 on 11/27/97

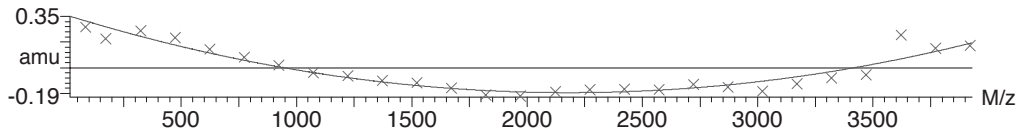
MS1 Static Calibration

28 matches of 28 tested references. SD = 0.0465



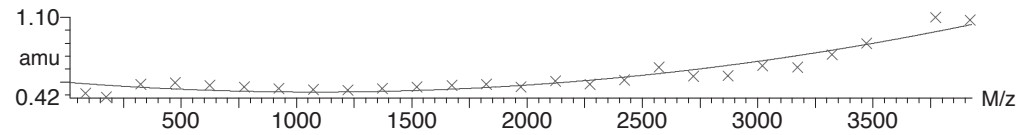
MS1 Scanning Calibration

27 matches of 28 tested references. SD = 0.0459



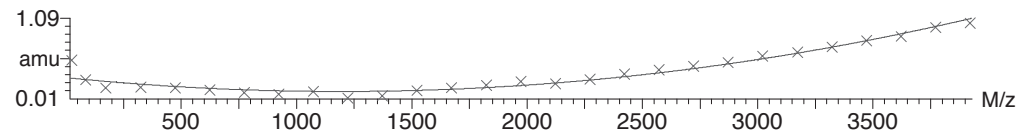
MS1 Scan Speed Compensation Calibration

26 matches of 28 tested references. SD = 0.0538



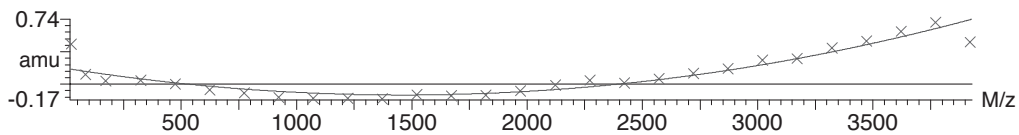
MS2 Static Calibration

28 matches of 28 tested references. SD = 0.0606



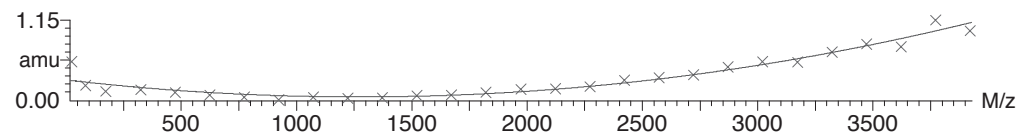
MS2 Scanning Calibration

28 matches of 28 tested references. SD = 0.0832



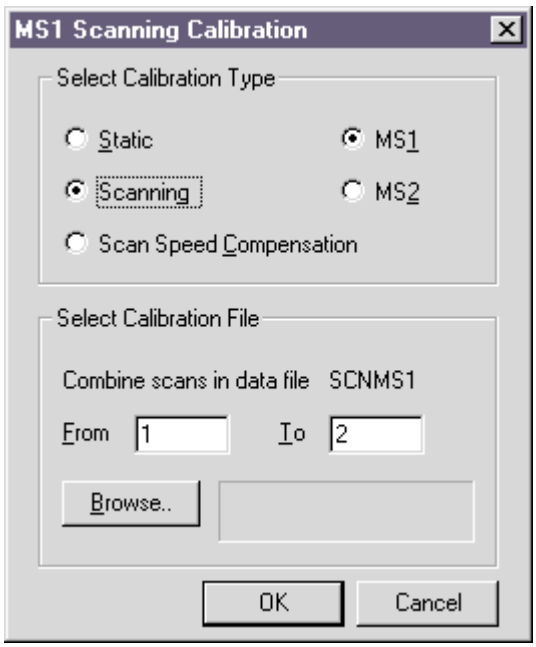
MS2 Scan Speed Calibration

28 matches of 28 tested references. SD = 0.0748



Checking the Calibration

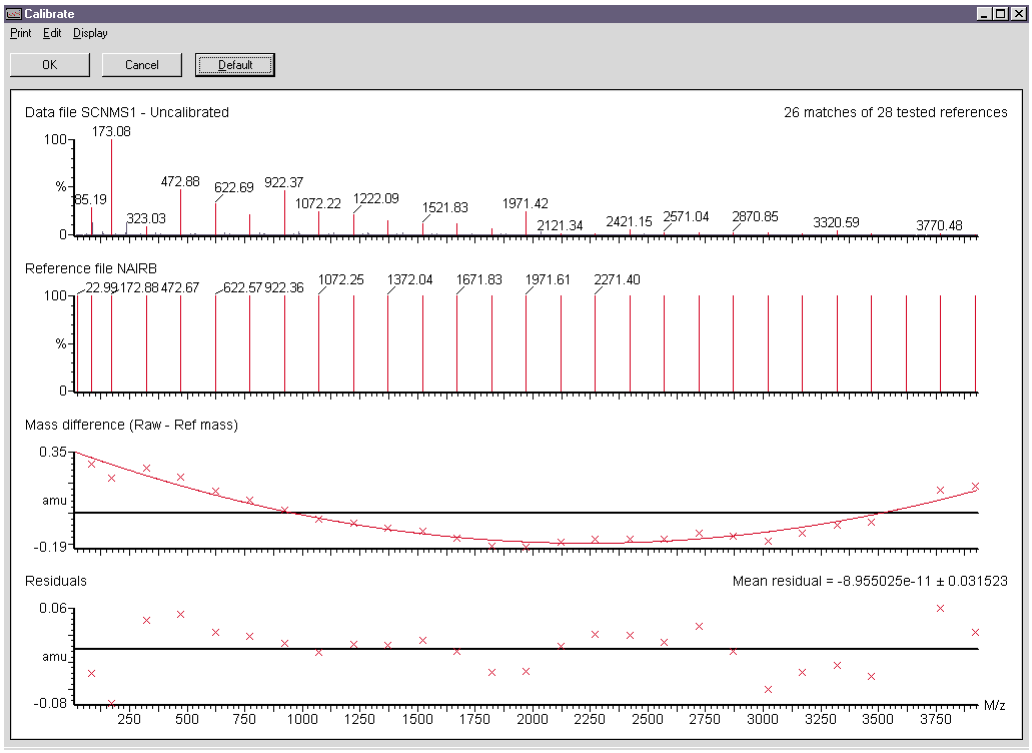
The calibration (successful or failed) can be viewed in more detail by selecting **Process**, **Calibration From File...** from the Calibrate dialog box. The dialog box which is then displayed (see below) allows the choice of calibration type for viewing. With the required calibration selected the correct calibration file is automatically called up.

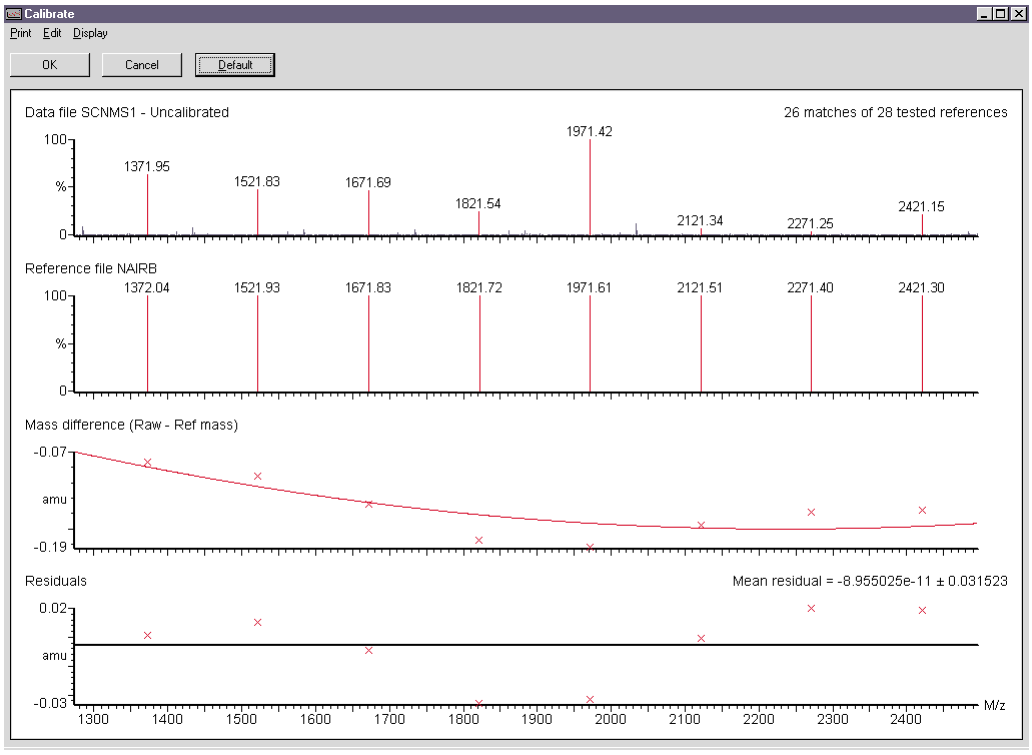


Clicking on the **OK** button repeats the calibration procedure for that particular file and display a calibration report on the screen. This calibration report (opposite upper) contains four displays:

- the acquired spectrum
- the reference spectrum
- a plot of mass difference against mass (the calibration curve)
- a plot of residual against mass

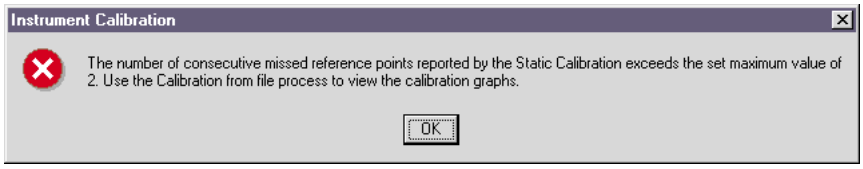
An expanded region can be displayed (opposite lower) by clicking and dragging with the left mouse button. In this way the less intense peaks in the spectrum can be examined to check that the correct peaks have been matched. The peaks in the acquired spectrum which have been matched with a peak in the reference spectrum are highlighted in a different colour.


Calibrate
Print Edit Display
OK Cancel Default
Data file SCNMS1 - Uncalibrated
26 matches of 28 tested references
173.08
85.19
323.03
472.88
622.69
922.37
1072.22
1222.09
1521.83
1971.42
2121.34
2421.15
2571.04
2870.85
3320.59
3770.48
Reference file NAIRB
22.99 172.88 472.67 622.57 922.36
1072.25
1372.04
1671.83
1971.61
2271.40
Mass difference (Raw - Ref mass)
0.35
amu
-0.19
Residuals
Mean residual = -8.955025e-11 ± 0.031523
0.06
amu
-0.08
250 500 750 1000 1250 1500 1750 2000 2250 2500 2750 3000 3250 3500 3750
M/z


Calibrate
Print Edit Display
OK Cancel Default
Data file SCNMS1 - Uncalibrated
26 matches of 28 tested references
1371.95
1521.83
1671.69
1821.54
1971.42
2121.34
2271.25
2421.15
Reference file NAIRB
1372.04
1521.93
1671.83
1821.72
1971.61
2121.51
2271.40
2421.30
Mass difference (Raw - Ref mass)
-0.07
amu
-0.19
Residuals
Mean residual = -8.955025e-11 ± 0.031523
0.02
amu
-0.03
1300 1400 1500 1600 1700 1800 1900 2000 2100 2200 2300 2400
M/z

Calibration Failure

If the calibration statistics do not meet the requirements then a message will be displayed describing at what point and why the calibration failed (an example of a failed calibration message is shown below). This message also states where the attempted calibration data can be viewed so that the exact cause of failure can be determined.


Instrument Calibration
The number of consecutive missed reference points reported by the Static Calibration exceeds the set maximum value of 2. Use the Calibration from file process to view the calibration graphs.
OK

There are a number of reasons for a calibration to fail:

- No peaks. If the acquired calibration data file contains no peaks the calibration will fail. This may be due to:

  Lack of reference compound.

  No flow of solvent into the source.

  Multiplier set too low.

- Too many consecutive peaks missed. If the number of consecutive peaks which are not found exceeds the **Missed Reference Peaks** parameter set in the Automatic Calibration Check, then the calibration will fail. Peaks may be missed for the following reasons:

  The reference solution is running out so that the less intense peaks are not detected.

  Multiplier is too low so that the less intense peaks are not detected.

  An incorrect ionisation mode is selected. Check that the data have been acquired with **Ion Mode** set to **ES+**.

  Note that it is possible to calibrate in negative ion mode electrospray using the naineg.ref reference file with a suitable reference solution.

  Intensity threshold, set in the Calibration Parameters dialog box, is too high. Peaks are present in the acquired calibration file but are ignored because they are below the threshold level.

  Either **Initial error** or **Peak window**, set in the Calibration Parameters dialog box, is too small. The calibration peaks lie outside the limits set by these parameters.

  Maximum Std Deviation, set in the Automatic Calibration Check dialog box, has been exceeded.

The wrong reference file has been selected. Check that the correct file (nairb.ref in this case) is selected in the Calibrate dialog box.

In the case of too many consecutive peaks missed:

Check the data in the on-screen calibration report to see if the missed peaks are present in the acquired calibration file.

If the peaks are not present then the first three reasons above are likely causes.

If the peaks are present in the data but are not recognised during calibration then the latter four are likely reasons.

Having taken the necessary action, proceed as follows:

If **Intensity threshold**, **Initial error** and **Peak window** are adjusted to obtain a successful calibration, check the on-screen calibration report to ensure that the correct peaks have been matched.

With a very low threshold and wide ranges set for the initial error and peak window it may be possible to select the wrong peaks and get a "successful" calibration. This is particularly relevant for calibrations with PEG where there may be peaks due to PEG+H^+, PEG+NH_4^+, PEG+Na^+, and also doubly charged species.

Select **OK** from the calibration report window to accept the new calibration, or select **Cancel** to retain the previous calibration.

Incorrect Calibration

If the suggested calibration parameters are used, and providing that good calibration data have been acquired, then the instrument should be calibrated correctly. However in some circumstances it is possible to meet the calibration criteria without matching the correct peaks. This situation is unusual, but it is always sensible to examine the on-screen calibration report to check that the correct peaks have been matched. These errors may occur when the following parameters are set:

- **Intensity threshold** set to 0
- **Initial error** too high (>2.0)
- **Peak window** too high (>1.5)
- **Maximum Std Deviation** too high (>0.2).

If the acquired spectrum looks like the reference spectrum and all of the expected peaks are highlighted then the calibration is OK.

An alternative cause of incorrect calibration is from contamination or background peaks. If a contamination or background peak lies within one of the peak matching windows, and is more intense than the reference peak in that window, then the wrong peak will be selected. Under some conditions this may happen with PEG. There are two ways to counter this:

- If the reference peak is closer to the centre of the peak window then the peak window can be narrowed until the contamination peak is excluded. Take care to ensure that no other reference peak is excluded.
- If the reference peak is not closer to the centre of the peak window, or if by reducing the window other reference peaks are excluded, then the calibration can be edited manually.

Manual Editing of Peak Matching

If an incorrect peak has been matched in the calibration process, this peak can be excluded manually from within the on-screen calibration report.

Using the mouse place the cursor over the peak in the acquired spectrum and click with the right mouse button.

The peak is excluded and is no longer highlighted.

If the true reference peak is present then this can be included in the calibration by the same procedure.

Place the cursor over the required peak and click with the right mouse button.

The peak is matched with the closest peak in the reference spectrum.

Manually editing one peak will not affect the other matched peaks in the calibration.

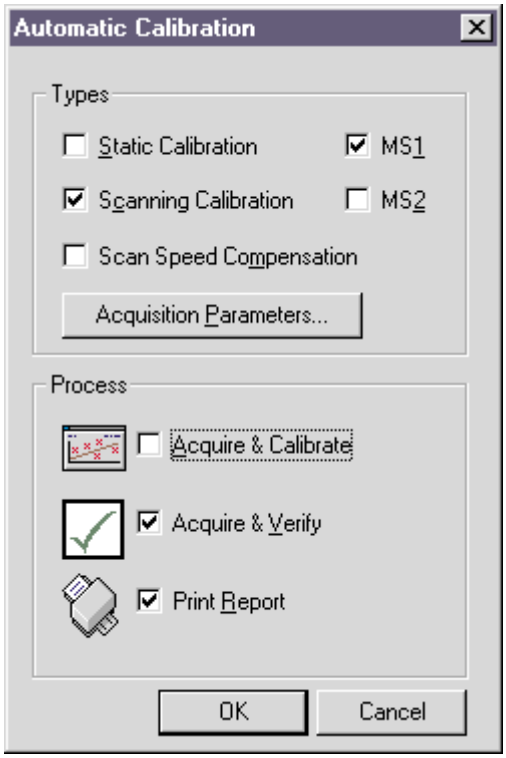
Saving the Calibration

When the instrument is fully calibrated the calibration can be saved under a file name so that it can be recalled for future use.

The recalled calibration has the same constraints of mass range and scan speed. The ion energy and resolution settings used for the calibration acquisition are also recorded as these can have an effect on mass assignment.

Verification

Once a full instrument calibration is in place it is not always necessary to repeat the full calibration procedure when the instrument is next used. Instead a calibration verification can be performed. (There is no benefit in verifying each calibration individually, re-calibration is just as quick.)



If a scanning acquisition is to be made and the calibration is to be checked:

Set up the instrument and access the calibrate dialog box as though a full calibration is to be carried out.

Set all peak matching parameters to the values that were used for the calibration.

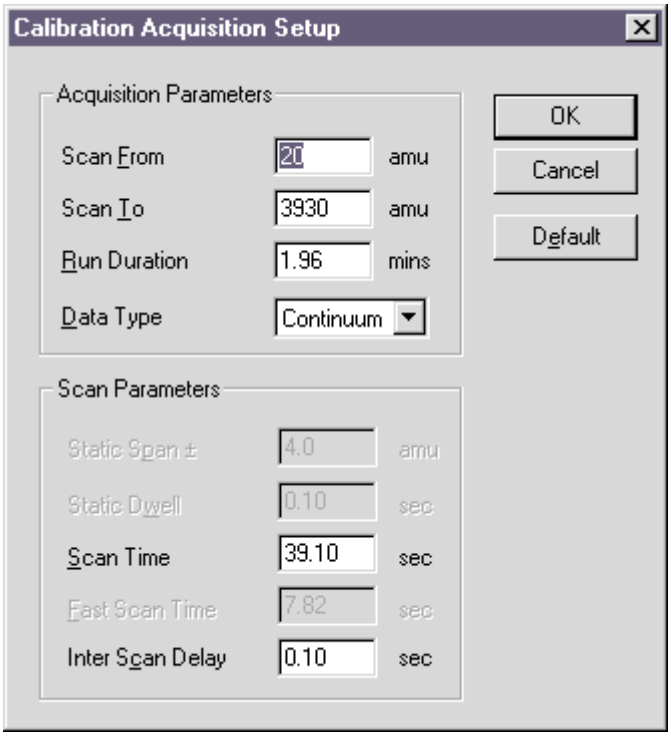
Bring up the Automatic Calibration dialog box by selecting **Start...** on the Calibrate dialog box.

Select **Scanning Calibration** and deselect **Static Calibration** and **Scan Speed Compensation**.

Deselect from **Acquire & Calibrate** and select **Acquire & Verify** and **Print Report.**

Select either MS1 or MS2, depending on the type of acquisition to be performed.

Select the **Acquisition Parameters ...** button to call up the Calibration Acquisition Set-up dialog box, as shown.



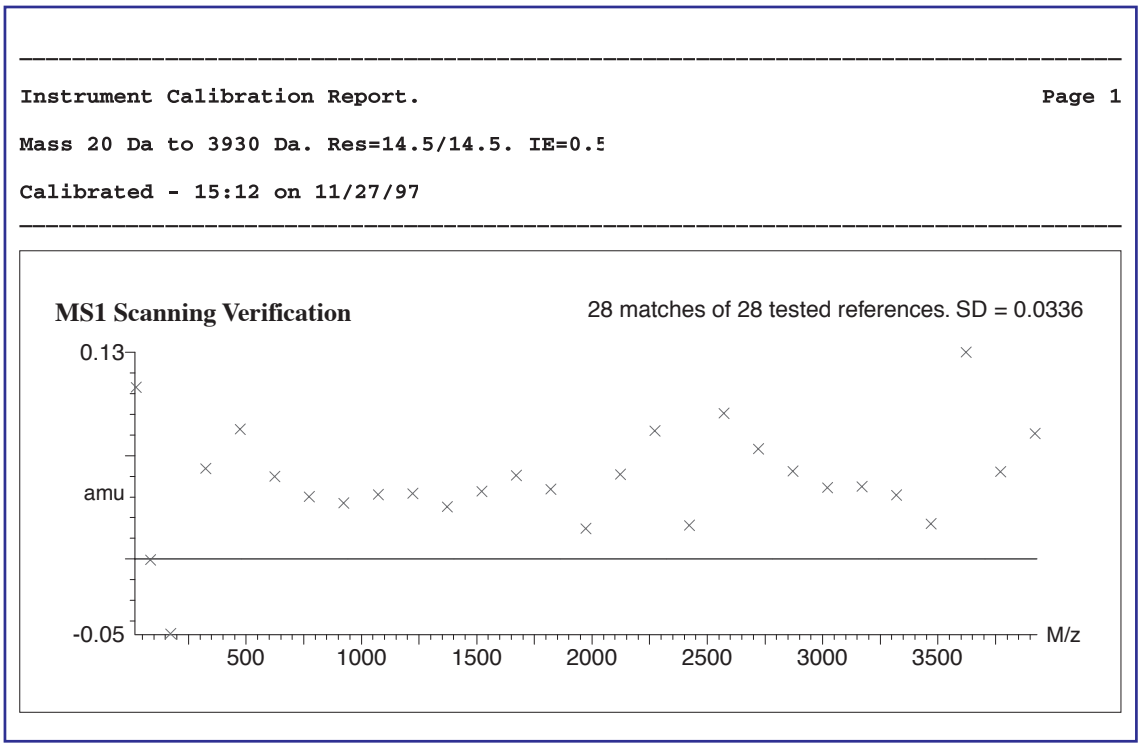
Set **Scan From**, **Scan To**, **Run Duration**, **Data Type**, **Scan Time** and **Inter Scan Delay** to agree with the acquisition parameters that are to be used for data acquisition.

With only the scanning calibration selected all of the other options in this dialog box are unavailable.

Select **OK** to return to the previous dialog box and **OK** again to start the verification procedure.

A scanning acquisition is now performed. When the acquisition is complete the data are combined to give a single spectrum which is compared against the reference file. A calibration curve is drawn and a report printed in a similar way to when the original calibration was performed. An example is shown below.

Unlike the original calibration procedure the instrument calibration is not changed and the report that is printed is a verification report.

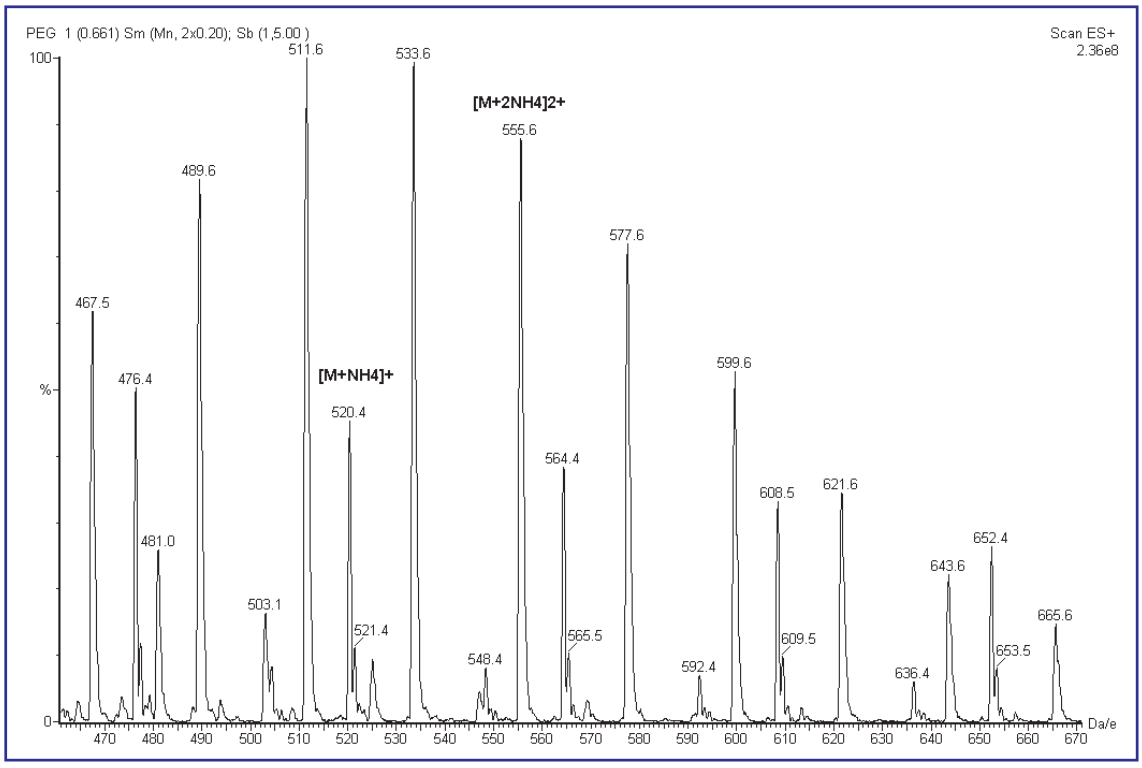


Electrospray Calibration with PEG

Caution should be used when calibrating with PEG in electrospray mode due to the number of peaks which are produced. Although ammonium acetate is added to the PEG reference solution to produce $[M+NH_4]^+$ ions, under some conditions it is quite usual to see $[M+H]^+$, $[M+Na]^+$ and doubly charged ions.

The spectrum shown below demonstrates how the PEG spectrum can be dominated by doubly charged ions (in this case $[M+2NH_4]^{2+}$) if the wrong conditions are chosen. In this case the concentration of ammonium acetate in the reference solution is too high (5mmol ammonium acetate is the maximum that should be used) and **Cone** is too low.

A low **Cone** voltage encourages the production of doubly charged ions. The voltage should be at least 30V.



Doubly charged peaks can be identified because the ^{13}C isotope peak is separated from the ^{12}C isotope by only 0.5 Da/e. If the instrument is set to unit mass and data is acquired in continuum mode the doubly charged peaks will appear broader as the isotopes will not be resolved.

Atmospheric Pressure Chemical Ionisation

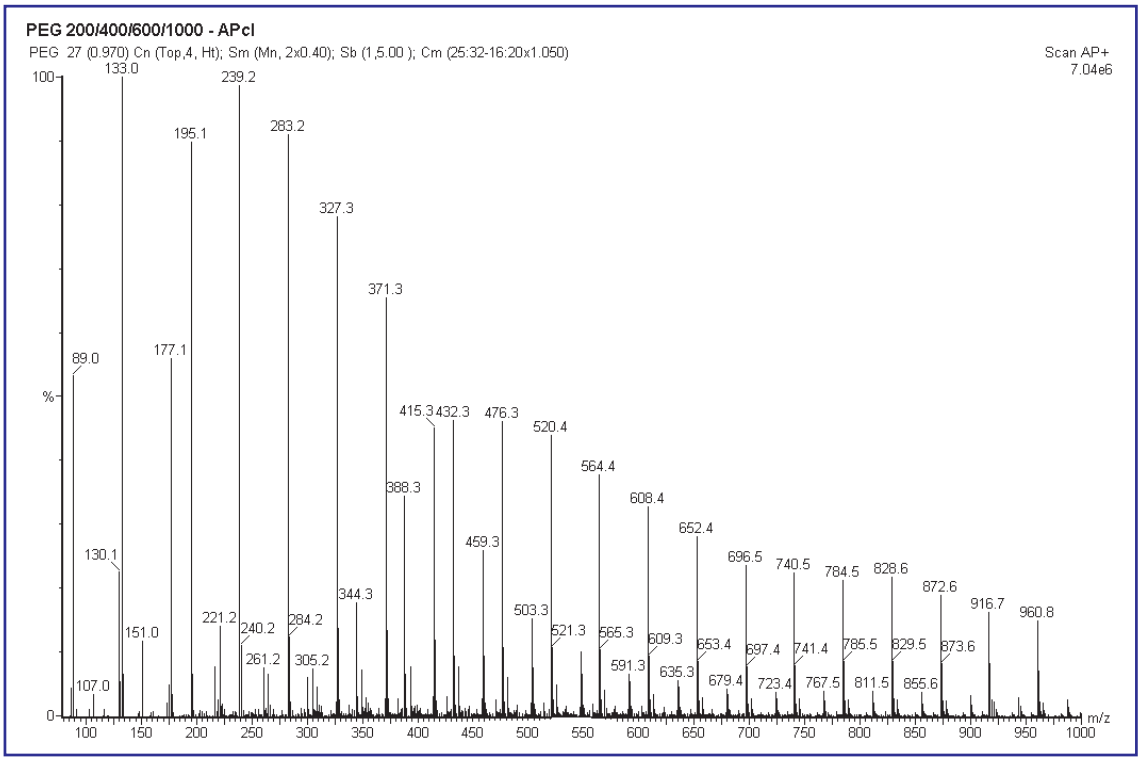
Introduction

This chapter describes a complete mass calibration of Quattro LC using atmospheric pressure chemical ionisation. The procedures described should be followed only after reading the previous chapter in this manual, describing the automated calibration with electrospray ionisation.

Due to the high flow rates used with APcI, the residence time of an injection of reference solution in the source is too short to allow a fully automated calibration, and the procedure therefore has to be carried out in several steps.

The recommended reference compound for APcI is a solution of polyethylene glycol (PEG) containing ammonium acetate. See *Reference Information* for advice on preparing the reference solution. See the following illustration for a typical PEG + NH_4^+ spectrum.

With PEG the possible calibration range is dependent upon the molecular weight distribution of the PEGs used in the reference solution. For this example PEG grades from PEG 200 to PEG 1000 are used.



Preparing for Calibration

Reference Compound Introduction

It is best to use a large volume injection loop (50µl) with a solvent delivery system set up to deliver 0.2 ml/min of 50:50 acetonitrile:water or methanol:water through the injector and into the APcI source. An injection of 50µl of reference solution lasts for approximately 15 seconds, allowing enough time to perform a slow scanning calibration.

Tuning

Before beginning calibration:

Set **Multiplier** to 650V.

Adjust source and lens parameters to optimise peak intensity and shape.

Set **Cone** in the region of 30-35V so that some fragmentation occurs to give some of the lower mass peaks in the spectrum.

Set the resolution and ion energy parameters for unit mass resolution on MS1 and MS2.

When a full calibration is completed it is possible to acquire data over any mass range within the calibrated range. It is therefore sensible to calibrate over a wide mass range and in this example the calibration will cover up to 1000 amu.

Calibration Options

To access the calibration options click on the **Calibrate** button of the acquisition control panel.

Selecting Reference File

Set **pegh1000.ref** as the reference file by clicking on the arrow in the reference file box and scrolling through the files until the appropriate file can be selected.

Leave the **Use Air Refs** box blank when calibrating in APcI.

Removing Current Calibrations

Select **uncal.cal** from the **File, Load Calibration...** menu option.

Select **Process**, **Delete all calibration...** followed by **File**, **Save Calibration**.

This ensures that a file with no calibration is currently active on the instrument and prevents any previously saved calibrations from being modified or overwritten.

Selecting Calibration Parameters

A number of parameters needs to be set before a calibration is started. Most of these parameters can be set at the same value as for electrospray. However, a **Polynomial order** of 2 is recommended for the calibration **Curve Fit**.

Performing a Calibration

The three types of calibration (static, scanning and scan speed) must be carried out in single steps.

Static Calibration

Access the Automatic Calibration dialog box by selecting **Start...** from the Calibrate page.

Check **Static Calibration** and **MS1** in the **Types** area of the dialog box.

In the **Process** area of the dialog box, check **Acquire & Calibrate**.

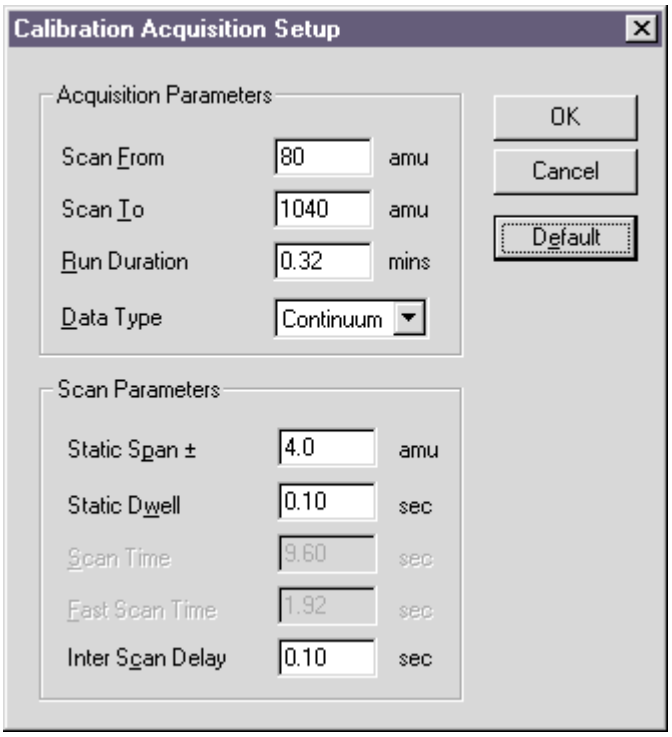
Acquisition Parameters

Selecting the **Acquisition Parameters...** button brings forward the default mass ranges, scan speeds and acquisition mode relevant to the pegh1000.ref reference file.

The upper area contains the **Acquisition Parameters** where mass range, run time and data type are set. When the instrument is fully calibrated any mass range or scan speed is allowed within the upper and lower limits dictated by the calibrations. It is therefore sensible to calibrate over a wide mass range. Since the pegh1000.ref reference file has peaks from m/z 63 to m/z 987, it is possible to calibrate over this mass range which is sufficient for the majority of applications with APcI. The following example shows a setup to achieve this.

Run Duration sets the time spent acquiring data for the static calibration. The time set must allow chance to inject a volume of reference solution and acquire several scans.

Data Type allows a choice of centroided, continuum or MCA data to be acquired. For APcI, while either continuum or centroided data may be used, **Continuum** is recommended.



The lower area in the Calibration Acquisition Setup dialog box contains the **Scan Parameters**.

> *When an instrument acquires data for a static calibration it first examines the selected reference file for the expected reference masses. It then acquires data over a small mass span around the expected position of each peak. Thus the acquired data do not contain continuous scans, but each "spectrum" is made up of small regions of acquired data around each peak separated by blank regions where no data are acquired.*

Static Span sets the size of this small region around each reference peak. A value of 4.0 amu is typical.

Static Dwell determines how much time is spent acquiring data across the span. A value of 0.1 second is suitable.

Slow Scan Time and **Fast Scan Time** are not available when a static calibration alone is selected.

Select **OK** from the Calibration Acquisition Setup to return to the Automatic Calibration dialog box.

Acquiring Data

To start the acquisition:

Select **OK** from the Automatic Calibration dialog box.

The instrument acquires a calibration file ready for static calibration using the data file name STAT. While data are being acquired:

Inject the reference solution.

Once the data have been acquired the instrument attempts to produce a static calibration automatically. The data file contains only a few scans of the reference compound, the remaining scans being of background.

As the automatic calibration procedure combines all of the scans in the data file to produce a calibration spectrum, the resulting spectrum may be too weak to give a successful calibration. Whether the calibration is successful or failed, it is wise to check the calibration manually.

Manual Calibration

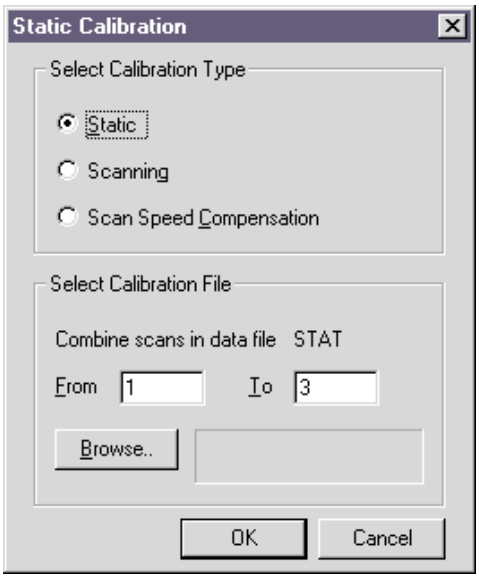
To perform a manual calibration using the acquired data:

From the chromatogram window call up the calibration file **STATMS1**.

Determine the scan numbers at the beginning and end of the chromatogram peak for the reference solution.

This can be achieved using **Process**, **Combine Spectra** *and using the left mouse button to drag across the peak. The start and end scans will be displayed in the combine spectra dialog box.*

Return to the Calibrate dialog box. Access the manual calibration options, as shown, by selecting **Process**, **Calibration From File...**.



Select **Static** calibration type and **MS1**.

In the lower area the data file **STATMS1** *should be selected automatically. If this is not the case the correct file can be selected by clicking on the* **Browse...** *button.*

Enter the start and end scans of the reference data in the **From** and **To** boxes.

Select **OK** to perform the calibration and display the calibration report on the screen (opposite upper).

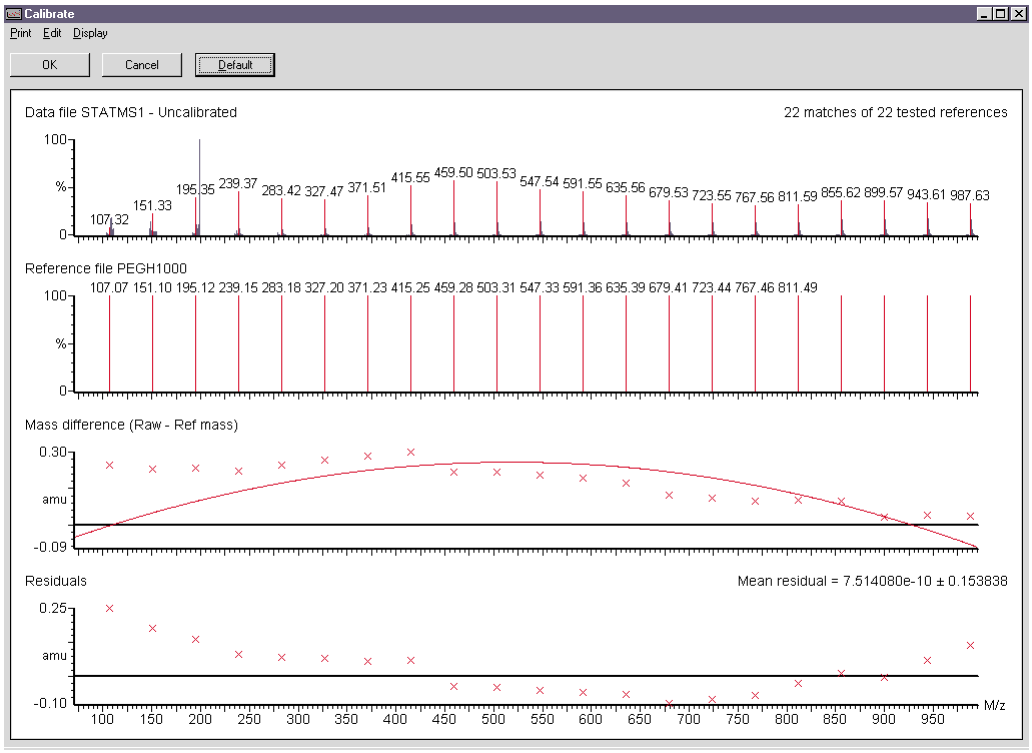
This report contains four displays:

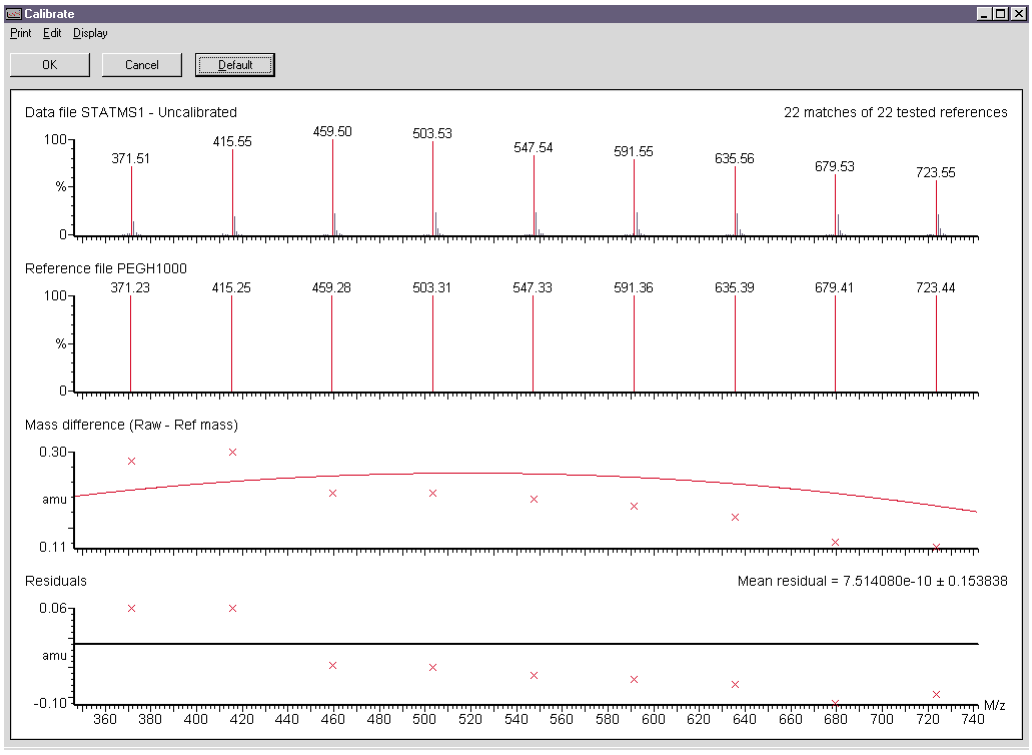
- the acquired spectrum
- the reference spectrum
- a plot of mass difference against mass (the calibration curve)
- a plot of residual against mass.

An expanded region (opposite lower) can be displayed by clicking and dragging with the left mouse button. In this way the less intense peaks in the spectrum can be examined to check that the correct peaks have been matched. The peaks in the acquired spectrum which have been matched with a peak in the reference spectrum are highlighted in a different colour.

Compare the acquired and reference spectra to ensure that the correct peaks have been matched.

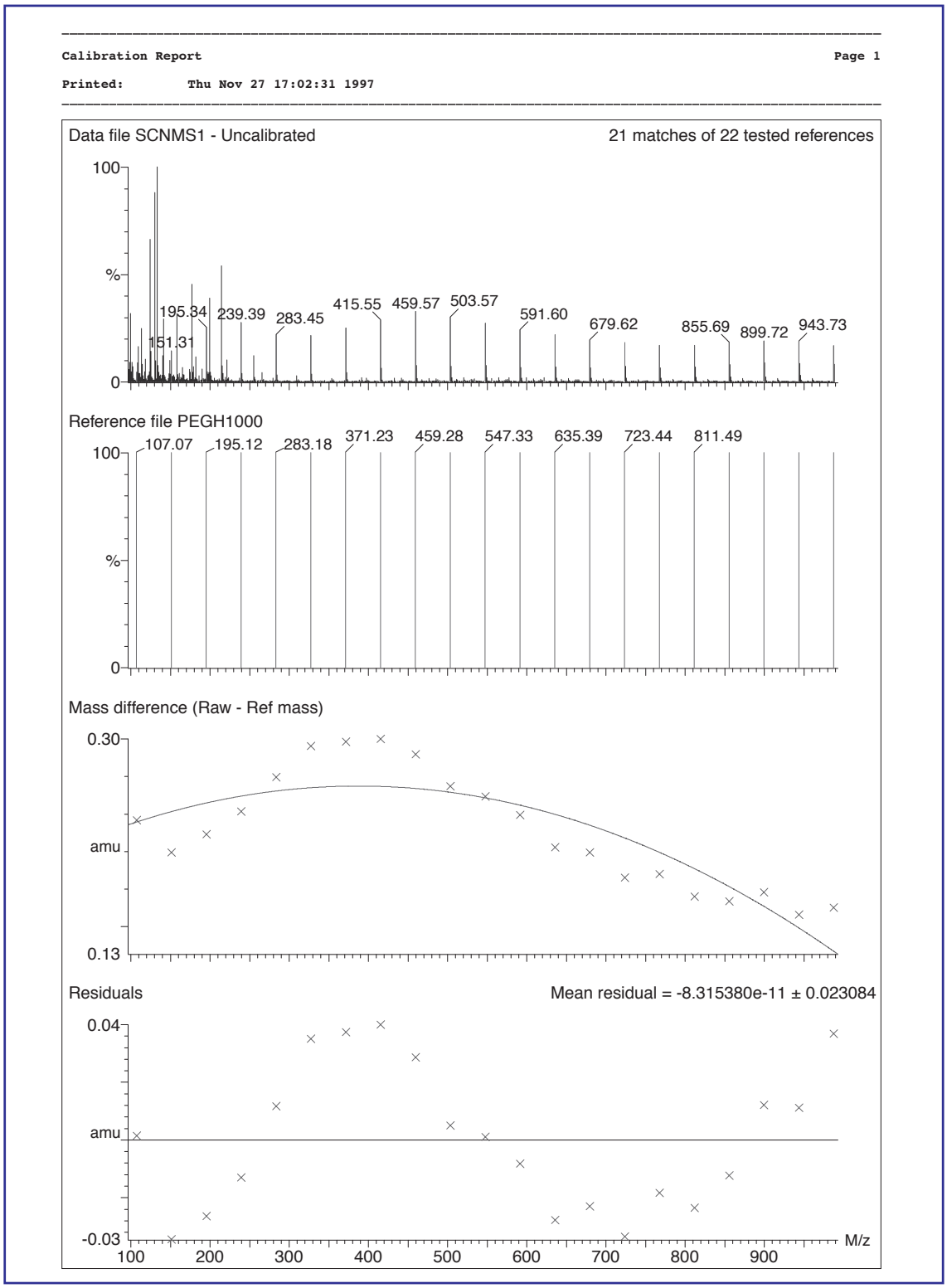
If insufficient peaks have been matched, or the wrong peaks have been matched, refer to the section on calibration failure later in this manual.


Calibrate
Print Edit Display
OK
Cancel
Default
Data file STATMS1 - Uncalibrated
22 matches of 22 tested references
107.32 151.33 195.35 239.37 283.42 327.47 371.51 415.55 459.50 503.53 547.54 591.55 635.56 679.53 723.55 767.56 811.59 855.62 899.57 943.61 987.63
Reference file PEGH1000
107.07 151.10 195.12 239.15 283.18 327.20 371.23 415.25 459.28 503.31 547.33 591.36 635.39 679.41 723.44 767.46 811.49
Mass difference (Raw - Ref mass)
Residuals
Mean residual = 7.514080e-10 ± 0.153838
M/z


Calibrate
Print Edit Display
OK
Cancel
Default
Data file STATMS1 - Uncalibrated
22 matches of 22 tested references
371.51 415.55 459.50 503.53 547.54 591.55 635.56 679.53 723.55
Reference file PEGH1000
371.23 415.25 459.28 503.31 547.33 591.36 635.39 679.41 723.44
Mass difference (Raw - Ref mass)
Residuals
Mean residual = 7.514080e-10 ± 0.153838
M/z

If the correct peaks have been matched then the report can be printed out:

Select **Print**, **Print** from the report display.



To accept the calibration:

Select **OK** from the calibration report.

Scanning Calibration and Scan Speed Compensation

Acquiring Data

To complete the calibration of the instrument two further data files must be acquired. Both files are acquired in scanning mode over the same mass range, one at the slowest speed required for scanning acquisitions and one at the fastest speed. Once these files have been acquired and used for calibration then data may be acquired anywhere within the mass range at any scan speed between the values used for the two sets of data. These data do not have to be acquired through the calibration dialog box, they can be acquired using the normal scan setup and then accessed from the calibration dialog box as described below.

The recommended scan speed for the scanning calibration is 100 amu/sec.

Set **Scan From** to 80 amu and **Scan To** to 1000 amu.

Set **Scan Time** to 9.2 sec and **Inter Scan Delay** to 0.1 sec.

Select **Continuum** as the **Data Type**.

Although Continuum is recommended centroided data may be used.

Set **Run Duration** to 2.0 minutes.

This allows time to start the acquisition, inject the reference solution and acquire several scans. With a solvent flow rate of 200 µl/min and a 50 µl loop in line, an injection of reference solution lasts approximately 15 seconds allowing at least one full scan of useful data to be acquired.

Choose any filename for the data.

The filename SCNMS1, the name used during an automatic calibration, is valid.

Start the acquisition and inject the reference solution.

The recommended scan speed for the scan speed compensation is 1000 amu/sec. This is the maximum scan speed permissible when using thresholded continuum data.

Although continuum is recommended centroided data may be used. It is possible to scan more quickly in centroided mode, but it is unlikely that a faster acquisition rate would be needed for general use.

Set **Scan From** to 80 amu and **Scan To** to 1000 amu.

Set **Scan Time** to 0.92 sec and **Inter Scan Delay** to 0.1 sec.

Select **Continuum** as the **Data Type**.

Set **Run Duration** to 2.0 minutes.

Choose any filename for the data.

The filename FASTMS1, the name used during an automatic calibration, is valid.

Start the acquisition and inject the reference solution.

Manual Calibration

Find the start and end scans of the reference data for each file in the same way as for the static calibration file.

From the Calibration dialog box select **Process**, **Calibration From File...**.

Select **Scanning** calibration type and **MS1**.

In the lower area the data filename **SCNMS1** *should be selected automatically. If this is not the case, or if an alternative filename has been used for the slow scanning acquisition, then the correct file can be selected by clicking on the* **Browse...** *button.*

Enter the start and end scans of the reference data in the **From** and **To** boxes.

Select the **OK** button to perform the calibration and display the calibration report on the screen in a similar way to the static calibration.

Compare the acquired and reference spectra to ensure that the correct peaks have been matched.

If the correct peaks have been matched then the calibration report can be printed out:

Select **Print, Print** from the report display.

If insufficient peaks have been matched or the wrong peaks have been matched see *Calibration Failure* later in this chapter. To accept the calibration:

Select **OK** from the calibration report.

The same procedure is used for the scan speed compensation except that **Scan Speed Compensation** is selected in the dialog box, and the fast scanning file is used. Note that for the scan speed compensation the default file is **FASTMS1**. If an alternative filename has been used then this must be selected using the data browser.

Once all three calibrations (static, scanning and scan speed compensation) have been completed then the instrument can be used for any mass range within the limits of the scanning calibrations and at any scan speed from 100 to 1000 amu/sec.

Calibrating MS2

The calibration of MS2 is carried out in exactly the same manner as above, except that data is acquired in MS2 mode instead of MS1.

Using the Instrument

Once all six calibrations (static, scanning and scan speed compensation, each for both MS1 and MS2) have been completed then the instrument can be used for any mass range within the limits of the scanning calibrations and at any scan speed from 100 to 1000 amu/sec.

Calibration Failure

When calibration is performed manually there is no warning message to show that the calibration has not met the set criteria. This must be judged by viewing the on-screen calibration report and examining the matched peaks and statistics associated with the report. There are a number of reasons for a calibration to fail:

- No peaks. If the acquired calibration data file contains no peaks the calibration has failed. This may be due to:

  Lack of reference compound.

  Wrong scans or wrong data file being used for the calibration.

  No flow of solvent into the source.

  Multiplier set too low.

- Too many consecutive peaks missed. If the number of consecutive peaks which are not found exceeds the limit set in the Automatic Calibration Check parameters then the calibration has failed. Peaks may be missed for the following reasons:

  The reference solution is running out causing less intense peaks to not be detected.

  Multiplier is too low and less intense peaks are not detected.

  The incorrect ionisation mode is selected. Check that the data has been acquired with **Ion Mode** set to **APcI+**.

  Intensity threshold, set in the Calibration Parameters dialog box, is too high. Peaks are present in the acquired calibration file but are ignored because they are below the threshold level.

  Either **Initial error** or **Peak window**, set in the Calibration Parameters dialog box, is too small. The calibration peaks lie outside the limits set by these parameters.

  Maximum Std Deviation (set in the Automatic Calibration Check dialog box) has been exceeded.

  The wrong reference file has been selected. Check that the correct file (peg1000.ref in this case) is selected in the Calibrate dialog box.

In the case of too many consecutive peaks missed:

Check the on-screen calibration report to see if the missed peaks are present in the acquired calibration file.

If the peaks are not present then the first three reasons above are likely causes.

If the peaks are present in the data, but are not recognised during calibration, then the latter four are likely reasons.

Having taken the necessary action, proceed as follows:

If **Intensity threshold**, **Initial error** and **Peak window** are adjusted to obtain a successful calibration, check the on-screen calibration report to ensure that the correct peaks have been matched.

With a very low threshold and wide ranges set for the initial error and peak window it may be possible to select the wrong peaks and get a "successful" calibration. This is particularly relevant for calibrations with PEG where there may be peaks due to PEG+H^+, PEG+NH_4^+ and PEG+Na. This situation is unusual, but it is always wise to examine the on-screen calibration report to check that the correct peaks have been matched.

Select **OK** from the calibration report window to accept the new calibration, or select **Cancel** to retain the previous calibration.

Incorrect Calibration

If the suggested calibration parameters are used and providing that good calibration data have been acquired, then the instrument normally calibrates correctly. However in some circumstances it is possible to meet the calibration criteria without matching the correct peaks.

This situation is unusual, but it is always wise to examine the on-screen calibration report to check that the correct peaks have been matched. These errors may occur when the following parameters are set:

- **Intensity threshold** set to 0
- **Initial error** too high (>2.0)
- **Peak window** too high (>1.5)
- **Maximum Std Deviation** too high (>0.2).

If the acquired spectrum looks like the reference spectrum and all of the expected peaks are highlighted then the calibration is OK.

An alternative cause of calibration failure is from contamination or background peaks. If a contamination or background peak lies within one of the peak matching windows, and is more intense than the reference peak in that window, then the wrong peak will be selected. Under some conditions this may happen with PEG. There are two ways to counter this:

- If the reference peak is closer to the centre of the peak window then the peak window can be narrowed until the contamination peak is excluded. Take care to ensure that no other reference peak is excluded.
- If the reference peak is not closer to the centre of the peak window, or if by reducing the window other reference peaks are excluded, then the calibration can be edited manually.

Manual Editing of Peak Matching

If an incorrect peak has been matched in the calibration process, this peak can be excluded manually from within the on-screen calibration report.

Using the mouse place the cursor over the peak in the acquired spectrum and click with the right mouse button.

The peak is excluded and is no longer highlighted.

If the true reference peak is present then this can be included in the calibration by the same procedure.

Place the cursor over the required peak and click with the right mouse button.

The peak is matched with the closest peak in the reference spectrum.

Manually editing one peak will not affect the other matched peaks in the calibration.

Saving the Calibration

When the instrument is fully calibrated the calibration can be saved under a filename so that it can be recalled for future use. For example, it is possible to save calibrations for use with different ionisation modes, so that when an ionisation source is switched the corresponding calibration is recalled.

The recalled calibration has the same constraints of mass range and scan speed. The ion energy and resolution settings used for the calibration acquisition are also recorded as these can have an effect on mass assignment.

Manual Verification

Once a full instrument calibration is in place it is not always necessary to repeat the full calibration procedure when the instrument is next used. Instead a calibration verification can be performed. (There is no benefit in verifying each calibration individually, re-calibration is just as quick.)

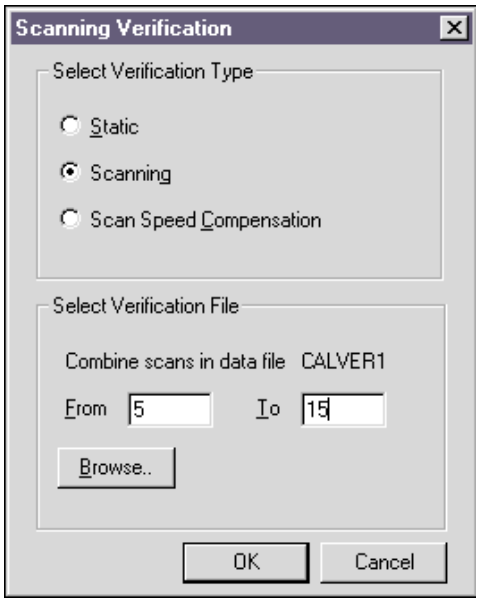
If a scanning acquisition is to be made and the calibration is to be checked:

Set up a scanning acquisition over the required mass range and at the required scan speed in the normal way.

Start the acquisition and inject the reference solution so that reference data is acquired.

Stop the acquisition.

Access the calibrate dialog box and set all peak matching parameters to the same values that were used for the calibration.

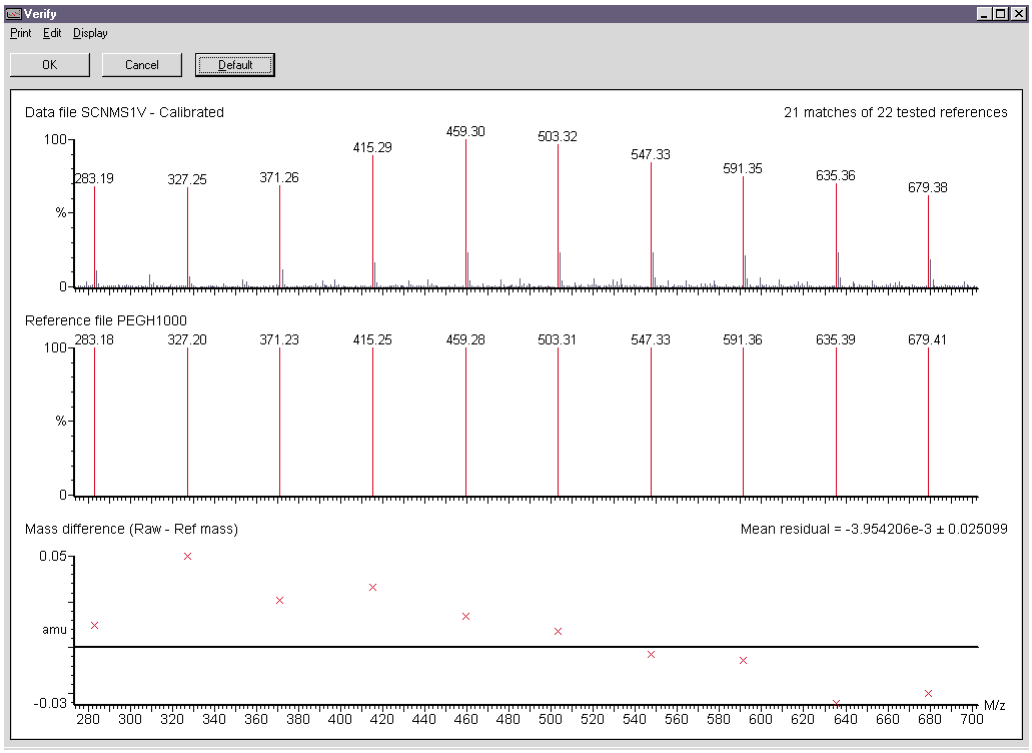


Select **Process**, **Verification from file...** and check **Scanning Calibration** (see below).

Select Scanning Calibration and either **MS1** or **MS2** depending on the type of data acquired.

Clicking on **Browse...**, select the acquired file and enter the start and end scans of the reference data.

Select **OK** to verify the calibration.



A calibration curve will be produced and displayed on the screen in a similar way to when the original calibration was performed. An example is shown above. When **OK** is selected from this report, unlike the original calibration procedure, the instrument calibration is not changed. As the verification procedure uses the same matching parameters as the calibration procedure, it is possible to validate the current calibration without re-calibrating the instrument.

The report can be printed out by selecting **Print**, **Print** from the verify report.

Maintenance and Fault Finding

Introduction

Cleanliness and care are of the utmost importance whenever internal assemblies are removed from the instrument.

- ✔ Always prepare a clear clean area in which to work.
- ✔ Make sure that any tools or spare parts that may be required are close at hand.
- ✔ Obtain some small containers in which screws, washers, spacers etc. can be stored.
- ✔ Use tweezers and pliers whenever possible.
- ✔ If nylon or cotton gloves are used take care not to leave fibres in sensitive areas.
- ✖ Avoid touching sensitive parts with fingers.
- ✖ Do not use rubber gloves.
- ✔ Before reassembling and replacing dismantled components, inspect O rings and other vacuum seals for damage. Replace with new if in doubt.

Should a fault occur soon after a particular part of the system has been repaired or otherwise disturbed, it is advisable first of all to ensure that this part has been correctly refitted and/or adjusted and that adjacent components have not been inadvertently disturbed.

Warning: Many of the procedures described in this chapter involve the removal of possibly toxic contaminating deposits using flammable or caustic agents. Personnel performing these operations should be aware of the inherent risks, and should take the necessary precautions.

Cooling Fans and Air Filters

Always ensure that none of the cooling fans is obstructed. It is essential that the fan filter is checked at regular intervals, and replaced if there is any doubt about its effectiveness.

The Vacuum System

The performance of the mass spectrometer will be severely impaired by the lack of a good vacuum in the ion transfer (hexapole) region or the analyser.

- An analyser pressure above 10^{-4} mbar results in a general loss in performance indicated by a loss of resolution and an increase in the background noise.
- Above 10^{-3} mbar the **Operate** and **Vacuum** LEDs on the instrument change from green to amber, indicating that the vacuum is insufficient to maintain the instrument in operate.
- Above 10^{-2} mbar the **Vacuum** LED changes to flashing red, indicating that the vacuum pump trips have been activated, followed by no indication when the instrument is no longer pumping.

Before suspecting a leak, the following points should be noted:

- The turbomolecular pumps will not operate if the rotary pump has failed.
- If the rotary pump is not maintained, the oil may become so contaminated that optimum pumping speed is no longer possible. Initially, gas ballasting may clean the oil. If the oil in the rotary pump has become discoloured, then it should be changed according to the pump manufacturer's maintenance manual.
- The turbomolecular pumps switch off if an over temperature is detected. This could be due to poor backing vacuum, failure of the water supply or a leak in the source or analyser.
- The turbomolecular pumps switch off if full speed is not achieved within a set time following start-up. This could be due to a leak or too high an ambient temperature.

Vacuum Leaks

If a leak is suspected, the following basic points may help to locate it:

- Leaks very rarely develop on an instrument that has been fully operational. Suspect components that have recently been disturbed.

  Leaks on flanges can usually be cured by further tightening of the flange bolts or by replacing the seal.

- All seals are made using O rings. When refitting flanges pay attention to the condition of O rings. Any that are cut or marked may cause a leak. The O rings should be clean and free from foreign matter.

  A hair across an O ring is sufficient to prevent the instrument pumping down.

- Source components that operate at, or slightly above, atmospheric pressure are not susceptible to vacuum leaks.

In the unlikely event of a leak on a feedthrough, then the unit should be replaced or returned to Micromass for repair.

Pirani Gauge

The Pirani gauge head does not require routine maintenance.

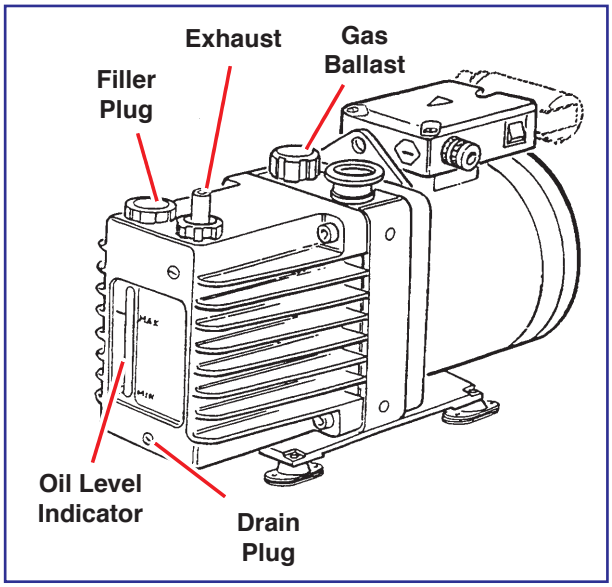
Active Inverted Magnetron Gauge

For information on cleaning the active inverted magnetron (Penning) gauge, refer to the Edwards literature supplied with the instrument.

Gas Ballasting

Gas ballasting serves two important purposes:

- When rotary pumps are used to pump away solvent vapours, the solvent vapour can become dissolved in the pump oil causing an increase in backing line pressure. Gas ballasting is a method of purging the oil to remove dissolved contaminants.
- Oil mist expelled from the rotary pump exhaust is trapped in the oil mist filter. This oil is returned to the rotary pump during gas ballasting.



Gas ballasting should be performed routinely on a weekly basis for 30 minutes. If the source is used in the APcI or megaflow electrospray modes, more frequent gas ballasting is recommended.

Gas ballasting is performed on the E2M28 pump by rotating the gas ballast valve 5 to 6 turns in a counterclockwise direction.

It is normal for the rotary pump to make more noise when the gas ballast valve is open.

Caution: Failure to gas ballast the rotary pump frequently leads to shortened oil lifetime which in turn may shorten rotary pump lifetime.

Caution: Under no circumstances should gas ballasting be performed during operation.

Oil Mist Filter

The E2M28 rotary pump is fitted with an Edwards EMF20 oil mist filter which traps oil vapour from the rotary pump exhaust. The trapped oil is then returned to the rotary pump during routine gas ballasting. The oil mist filter contains two elements which require the following maintenance:

- Change the odour element monthly or whenever the pump emits an oily odour.
- Change the mist element every time the rotary pump oil is changed.

To change the elements follow the instructions in the Edwards manual.

Foreline Trap

The foreline trap stops oil vapour migrating from the rotary pump to the mass spectrometer. During normal use, the activated alumina (sorbent) will absorb any oil vapour, becoming brown in colour. The sorbent should be replaced when this discolouration reaches the region of the trap furthest from the pump (the vacuum side). The manufacturers recommend that the sorbent is replaced routinely at three-monthly intervals.

With the instrument vented and the pump switched off, replace the sorbent as described in the manufacturer's literature.

Rotary Pump Oil

The oil in the rotary pump should be maintained at the correct level at all times. Check the oil level at weekly intervals, topping up if necessary.

It is important to monitor the condition of the oil regularly. Replace the oil when it has changed to a noticeable red colour, or routinely at 4 month intervals (3000 hours operation). At the same time, replace the oil mist filter's mist element (see above).

Change the oil in the rotary pump as follows:

Gas ballast lightly for 30 to 60 minutes.

Vent and shut down the instrument as described in *Routine Procedures*.

It will be found easier to drain the oil while the pump is still warm.

Drain the oil through the drain hole situated near the oil level sight glass.

Flush the pump, then replace the drain plug and refill the pump with the correct grade oil to the correct level.

Gas ballast lightly for 30 to 60 minutes.

For further servicing information refer to the manufacturer’s manual.

The Source

Overview

The Z-spray source is a robust assembly requiring little maintenance. The source consists of three basic parts:

- The probe adjustment flange.
- The glass tube.
- The source flange assembly.

The probe adjustment flange and the glass tube can be readily removed, without venting the instrument, to gain access to the source block and sample cone. This allows the following operations to be performed:

- Wiping the sample cone.
- Removing the sample cone.
- Fitting or removing the APcI discharge pin.
- Fitting or removing the exhaust liner and cleanable baffle.
- Fitting or removing the nanoflow electrospray interface.
- Enabling or disabling the purge gas.

The sample cone may be cleaned in situ, by gentle wiping with a cotton swab or lint tissue soaked with 50:50 acetonitrile:water. More thorough cleaning of the sample cone may be achieved by removing it from the source. This may also be done without venting the instrument, by closing the isolation valve located on the ion block. Less frequently it may be necessary to clean the ion block, the extraction cone and the hexapole lens, in which case the instrument must be vented. This should only be done when the problem is not rectified by cleaning the sample cone or when charging effects are apparent.

> *Charging is evidenced by a noticeable progressive drop in signal intensity, often resulting in a complete loss of signal. Switching the instrument out of and back into operate causes the beam momentarily to return.*

The hexapole transfer lens should not require frequent cleaning. If it is suspected that the lens does need cleaning it may be withdrawn from the front of the instrument after removing the ion block support.

Warning: Cleaning the various parts of the source requires the use of solvents and chemicals which may be flammable and hazardous to health. The user should take all necessary precautions.

Cleaning the Sample Cone in Situ

This may be necessary due to lack of sensitivity or fluctuating peak intensity, or if deposited material is visible on the outside of the sample cone. Proceed as follows:

On the MassLynx top-level window, click on to launch the tune page.

Deselect OPERATE.

Switch off the LC pumps.

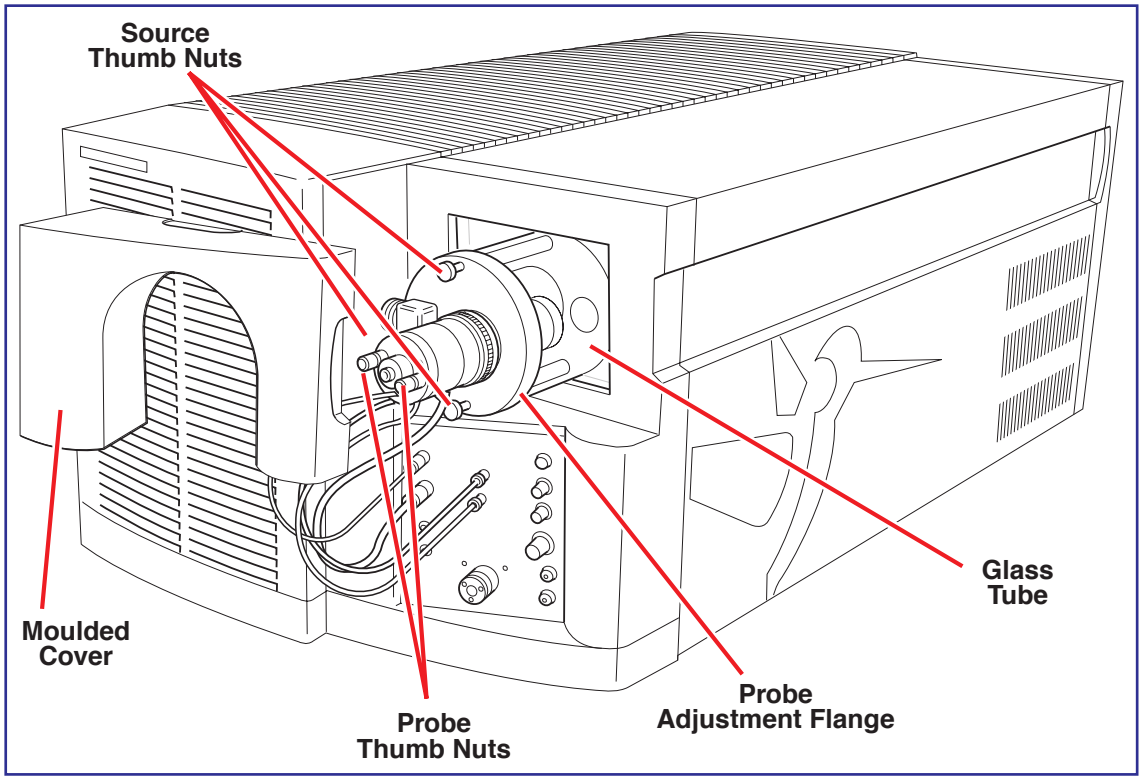
Disconnect the liquid flow at the rear of the probe.

Set **Source Block Temp** and either **APcI Probe Temp** or **Desolvation Temp** to 20°C to switch off the heaters.

Warning: Removal of the APcI probe or desolvation nozzle when hot may cause burns.

Caution: Removal of the APcI probe when hot will shorten the probe heater's life.

The cooling time will be significantly shortened if the API gases are left flowing.



When **APcI Probe Temp** or **Desolvation Temp** has cooled below 100°C:

Switch off the nitrogen supply by selecting **Gas** followed by **Nitrogen**.

Disconnect both gas lines from the front panel by undoing the knurled nuts.

Disconnect both electrical connections by pulling back on the plug sleeves to release the plugs from the sockets on the front panel.

Undo the two knurled thumb nuts that retain the probe and withdraw it from the source. Place it carefully to one side.

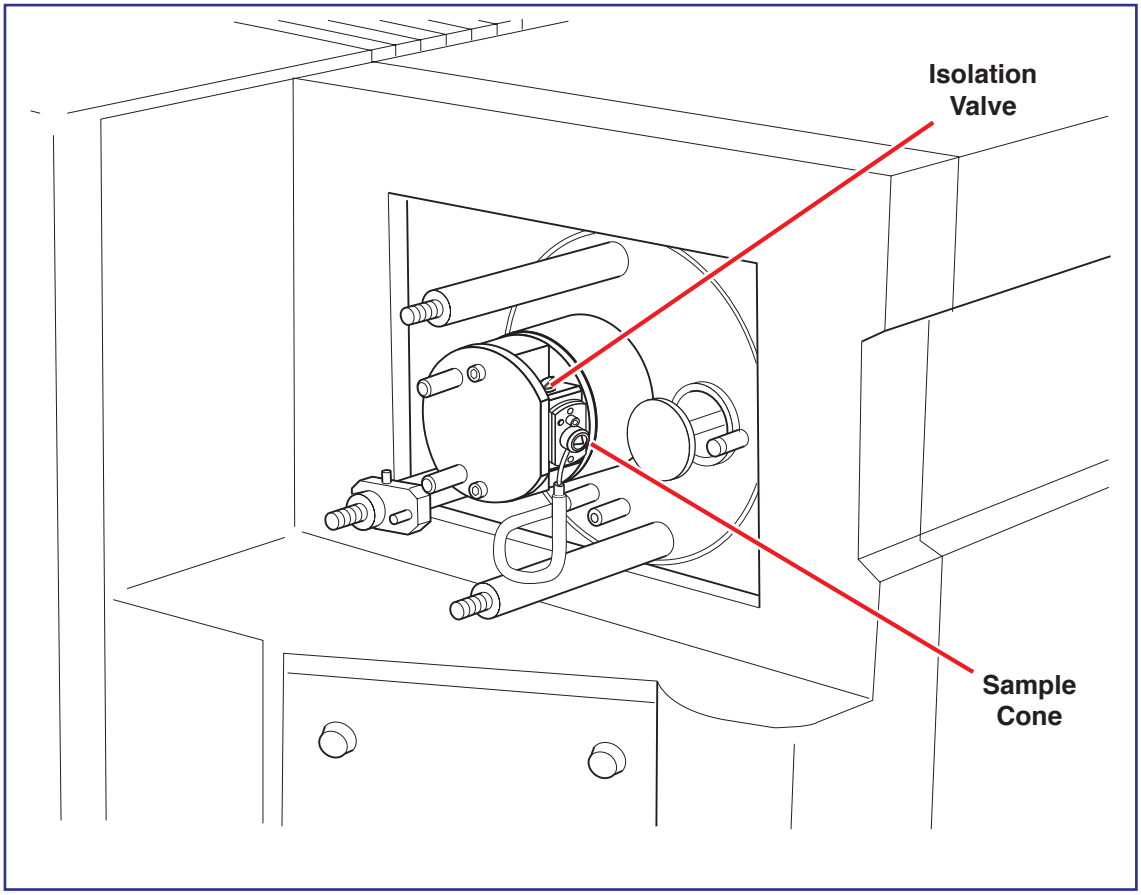
Remove the moulded cover from around the source.

Undo the three thumb screws and withdraw the probe adjustment flange and glass tube. Place the glass tube, end on, on a flat surface and place the probe adjustment flange on top of the glass tube.

Warning: When the source enclosure has been removed the source block is exposed. Ensure that the source block heater has cooled before proceeding.

If fitted, remove the APcI discharge pin.

The sample cone is now accessible.



Using a suitable flat blade screwdriver rotate the isolation valve by 90° into its fully anticlockwise position.

A small improvement in the analyser vacuum may be observed as a result of this operation.

The isolation valve is closed when the slot is perpendicular to the direction of flow.

Carefully wipe the sample cone with a cotton swab or lint free tissue soaked in 50:50 acetonitrile:water or 50:50 methanol:water.

Caution: Do not attempt to remove any obstruction by poking. This may result in damage to the sample cone.

Dry the cone using nitrogen.

If the sample cone is still not clean, or if the aperture is partially blocked, proceed to the following section. Otherwise, when the cone is clean and dry:

Open the isolation valve.

Replace all removed components, following in reverse order the removal procedures.

Removing and Cleaning the Sample Cone

Caution: The sample cone is a delicate and expensive component and should be handled with extreme care.

It is not necessary to vent the instrument to remove the sample cone. The source block incorporates an isolation valve for this purpose. To remove the sample cone proceed as follows:

Follow the procedure in the previous section, to gain access to the sample cone.

Using a suitable flat blade screwdriver rotate the valve by 90° into its fully anticlockwise position.

A small improvement in the analyser vacuum may be observed as a result of this operation.

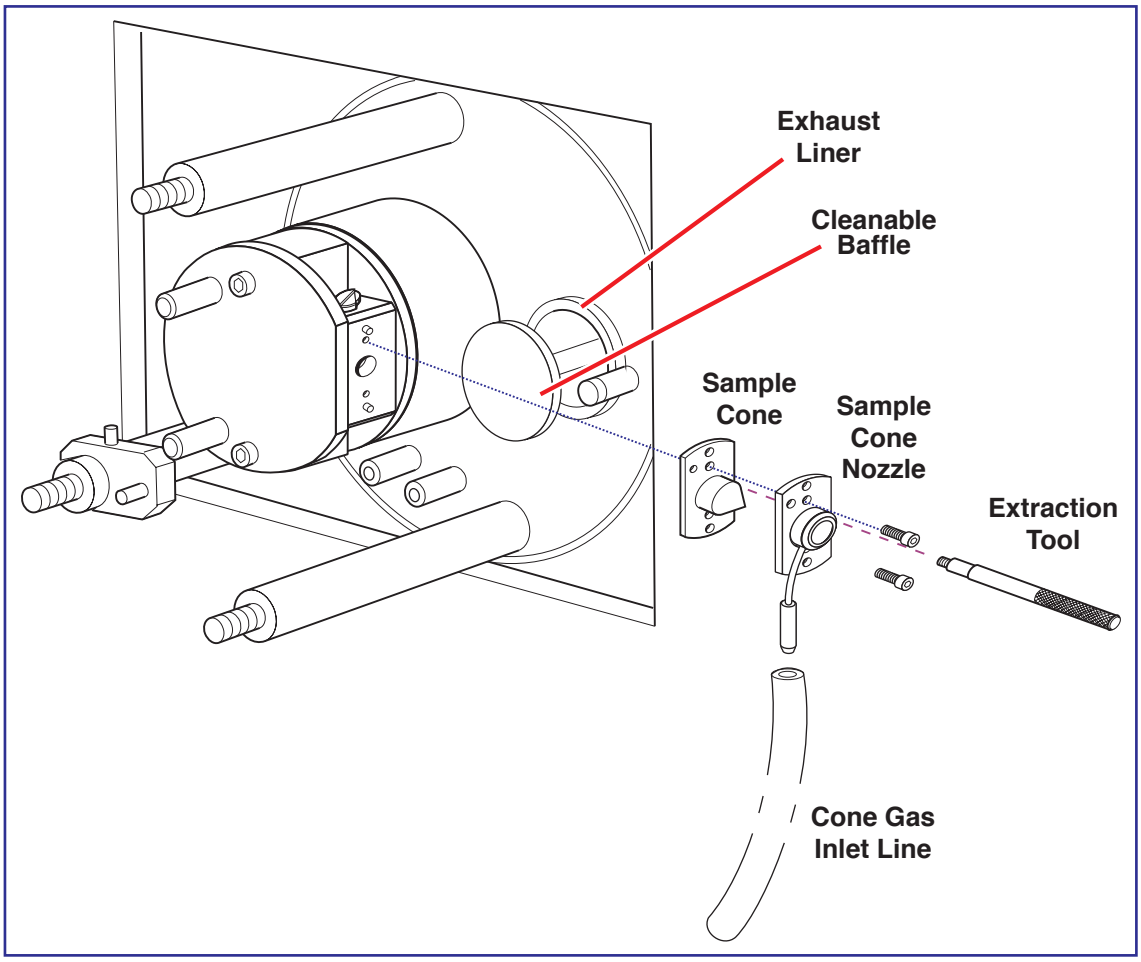
The isolation valve is in the closed position when the slot is perpendicular to the direction of flow.

Disconnect the cone gas inlet line (if fitted).

Take the sample cone extraction tool supplied in the source spares kit and screw it to the flange of the sample cone.

Remove the two sample cone retaining screws using a 1.5mm Allen key and withdraw the sample cone and sample cone nozzle (if fitted) from the ion block.

Remove the extraction tool, and separate the sample cone from the sample cone nozzle. Place both components in an ultrasonic bath containing 50:50 acetonitrile:water or 50:50 methanol:water.



Dry the cone and nozzle using nitrogen.

To minimise down time fit a spare sample cone, obtainable from Micromass, at this stage.

If material has built up on the exhaust liner and cleanable baffle:

Remove the cleanable baffle and the exhaust liner.

Clean these components, or obtain replacements.

Fit the cleaned (or the replacement) exhaust liner and cleanable baffle to the ion block.

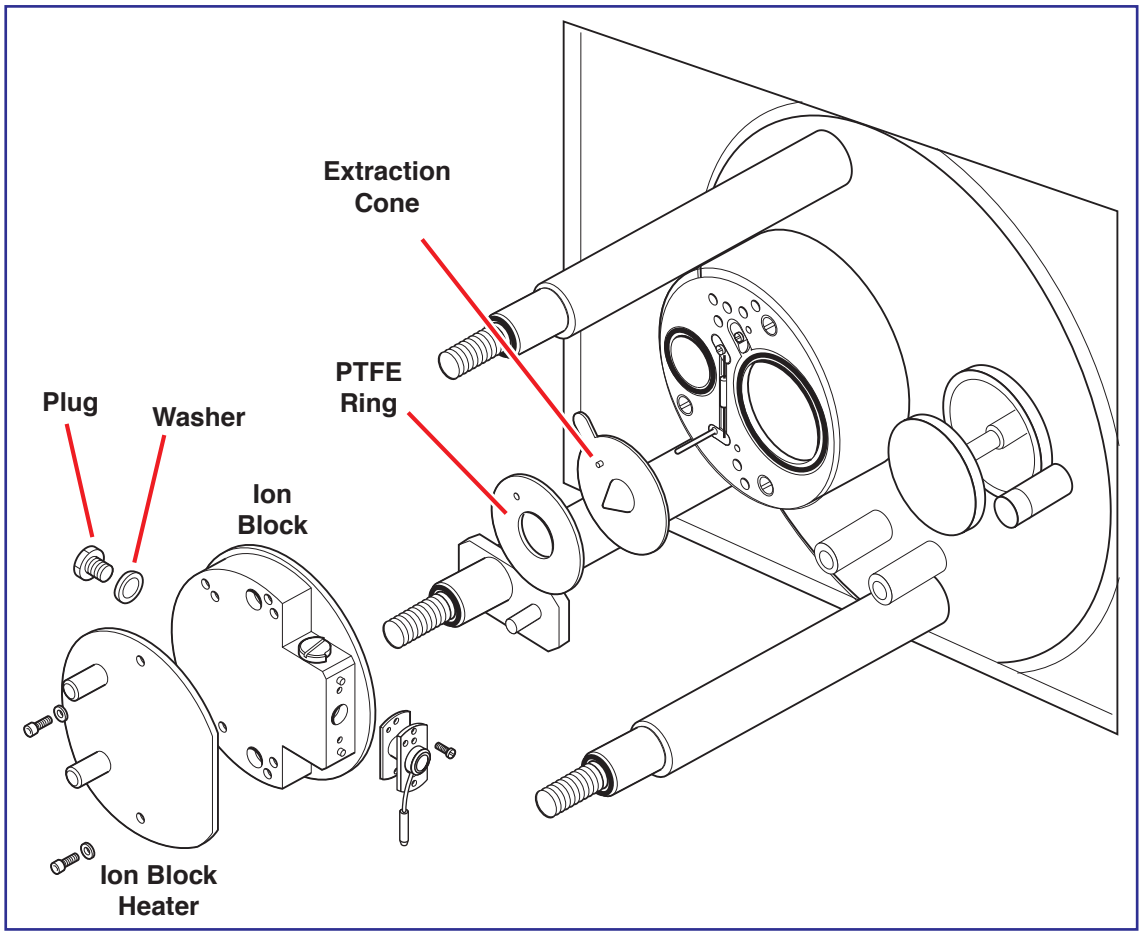
Refitting the sample cone is a reversal of the removal procedure.

Removing and Cleaning the Source Block and Extraction Cone

On the tune page select **Other** from the menu bar at the top of the tune page. Click on **Vent**.

The rotary pump and the turbomolecular pumps switch off. The turbomolecular pumps are allowed to run down to 50% speed after which a vent valve opens to atmosphere automatically.

Remove the source enclosure and the sample cone as described in the previous section.



When the instrument has vented:

Remove the two screws which secure the ion block and remove the ion block heater and the ion block.

Separate the extraction cone and the PTFE insulating ring from the ion block.

Remove the plug and the PTFE sealing washer.

Remove the sample cone as described above.

Leaving the valve stem in place, immerse the ion block in an ultrasonic bath containing 50:50 acetonitrile:water or 50:50 methanol:water, followed by 100% methanol.

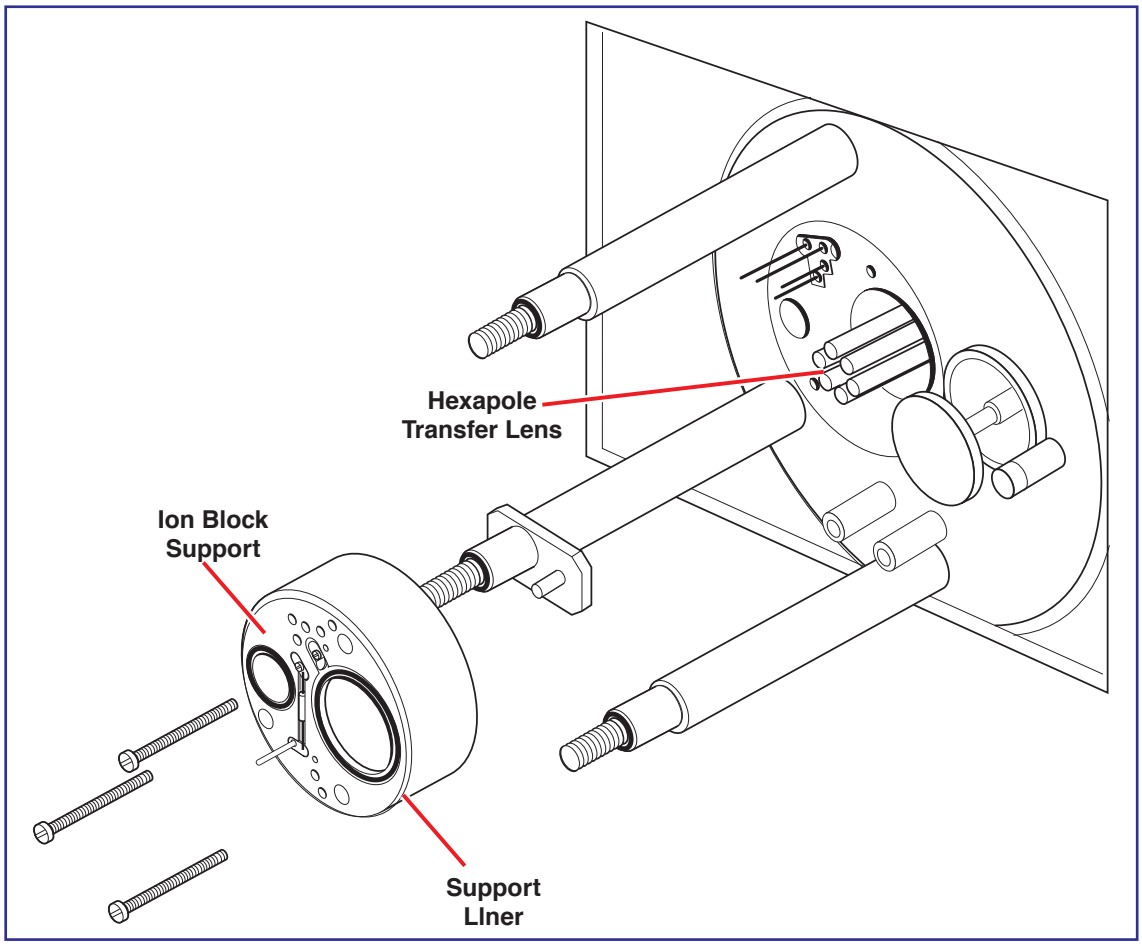
Clean the sample cone and the extraction cone using in turn:

- concentrated formic acid.
- 50:50 acetonitrile:water or 50:50 methanol:water.
- 100% methanol.

Warning: Strong acid causes burns. Carry out this procedure in a fume cupboard using protective equipment.

Dry all components using a flow of nitrogen, or place them in a warm oven.

Removing and Cleaning the Hexapole Transfer Lens Assembly



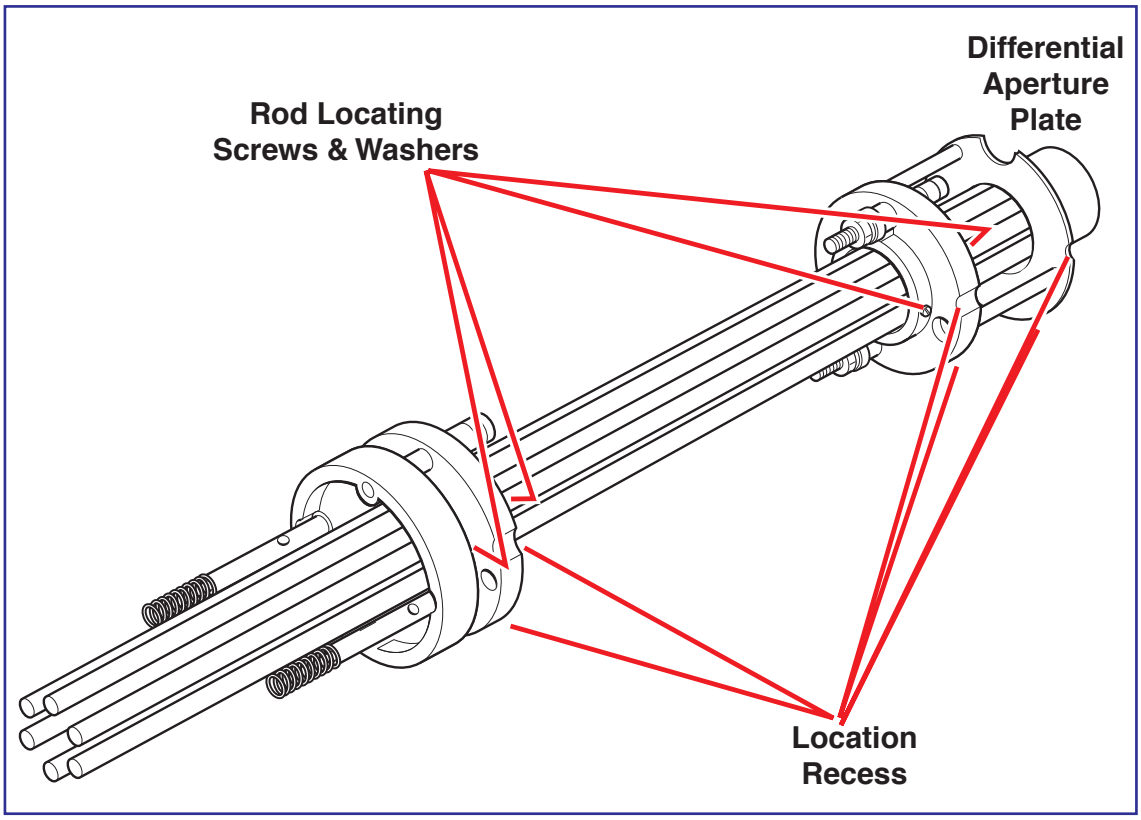
To remove the hexapole transfer lens assembly, proceed as follows:

Remove the ion block, as described above.

Remove the three screws retaining the ion block support and carefully withdraw it, together with the support liner and O rings, from the pumping block.

Using a lint free tissue to gently grasp the hexapole, carefully withdraw it.

Caution: Take care not to scratch the internal bore of the pumping block as the hexapole lens assembly is withdrawn.



To clean the hexapole transfer lens proceed as follows:

Immerse the complete assembly in a suitable solvent (100% methanol) and sonicate in an ultrasonic bath.

Thoroughly dry the assembly using a flow of nitrogen.

In severe cases:

Remove, clean, dry and replace each rod separately (one at a time).

Reassemble the assembly with extreme care, checking the assembly against the diagram.

Reassembling and Checking the Source

Feed the hexapole transfer lens into the instrument, allowing the recesses in the differential aperture plate to locate onto the two support rails within the analyser assembly. Ensure that the assembly is pushed fully in.

Check the condition of the O rings on the ion block support. Replace them if necessary.

Replace the ion block support, pushing it in against the springs of the hexapole assembly.

Replace the three retaining screws.

Fit the plug and sealing ring to the ion block.

Fit the insulating ring and extraction cone.

Offer the ion block up to the peek ion block support, locate the two dowels and push firmly.

Replace the ion block heater.

Replace and firmly tighten the two retaining screws taking care not to over-tighten the screws.

Check that the isolation valve is closed.

On the tune page select **Other** and click on **Pump**.

Replace the PTFE exhaust liner and cleanable baffle, if removed.

Replace the sample cone and, if the nanoflow option is to be used, the sample cone nozzle on the ion block.

Reconnect the cone gas supply (nanoflow operation only).

When the instrument has pumped down:

Open the isolation valve.

Plug the purge and cone gas outlets and fit the APcI corona discharge pin.

Fit the source enclosure and the probe adjustment flange.

Insert the APcI probe and connect the **Nebuliser** gas line.

Select **Gas** and turn on **Nitrogen**. Fully open the **Nebuliser** gas valve.

Set **Desolvation Gas** to read back 400 litres/hour (monitored on the tune page).

Check for gas leaks using soap solution.

Reduce **Desolvation Gas** to 150 litres/hour.

Set **Source Block Temp** to 150°C, and **APcI Probe Temp** to 20°C

Caution: The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Select OPERATE on the tune page.

With **Corona** set to zero, check that the **Cone** readback is reading the correct set value.

Set **Corona** to 4.0kV.

Check that the **Corona** readback is 4.0 kV and that the **Cone** readback is still reading the same set value.

Check that all other readbacks on the tune page agree with the set values.

The Discharge Pin

If the corona discharge pin becomes dirty or blunt:

Remove it from the source.

Clean and sharpen it using 600 grade emery paper.

If the needle becomes bent or otherwise damaged it should be replaced.

The Electrospray Probe

Overview

Indications that maintenance is required to the electrospray probe include:

- An unstable ion beam.

  Nebulising gas may be escaping from the sides of the probe tip.

  Ensure that the probe tip O ring is sealing correctly.

  The probe tip setting may be incorrect.

  Adjust the probe tip setting as described in *Electrospray*.

  The probe tip may be damaged.

  Replace the probe tip.

  There may be a partial blockage of the sample capillary or the tubing in the solvent flow system.

  Clear the blockage or replace the tubing.

- Excessive broadening of chromatogram peaks.

  This may be due either to inappropriate chromatography conditions, or to large dead volumes in the transfer capillaries between the LC column or probe connection.

  Ensure that all connections at the injector, the column, the splitting device (if used) and the probe are made correctly.

- High LC pump back pressure.

  With no column in line and the liquid flow set to 300 µl/min the back pressure should not exceed 7 bar (100 psi). Pressures in excess of this indicate a blockage in the solvent flow system.

  Samples containing particulate matter, or those of high concentrations, are most likely to cause blockages.

  Check for blockages at the tube connections and couplings to the injector, the column and, if used, the flow splitter.

  Concentrated formic acid can be injected to clear blockages. Rinse thoroughly afterwards.

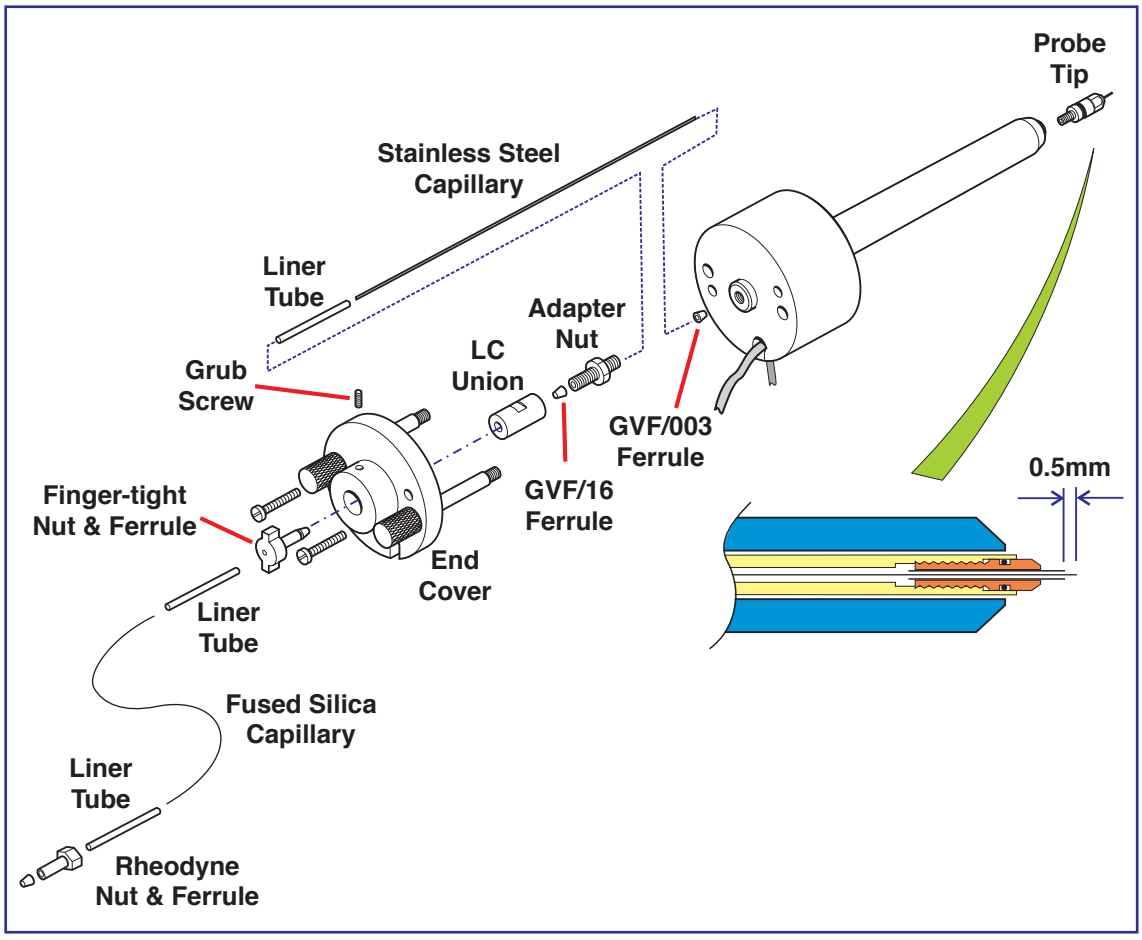
Blockage of the stainless steel sample capillary may occur if the desolvation heater is left on without liquid flow. This is particularly relevant for samples contained in involatile solvents or high analyte concentrations. To avoid this problem it is good practice to switch off the heater before stopping the liquid flow, and flush the capillary with solvent.

A blocked stainless steel sample capillary can often be cleared by removing it and reconnecting it in the reverse direction, thus flushing out the blockage.

- Gas flow problems

  Check all gas connections for leaks using soap solution, or a suitable leak searching agent such as Snoop.

Replacement of the Stainless Steel Sample Capillary



If the stainless steel sample capillary cannot be cleared, or if it is contaminated or damaged, replace it as follows:

Remove the probe form the source.

Disconnect the LC line from the probe and remove the finger-tight nut.

Loosen the grub screw retaining the LC union.

Remove the two probe end cover retaining screws, and remove the probe end cover.

Unscrew and remove the probe tip.

Remove the LC union and adapter nut. Withdraw and discard the stainless steel sample capillary.

Remake the LC connection to the LC union.

Sleeve one end of new sample capillary with the PTFE liner tube.

Using a GVF/16 ferrule and the adapter nut, connect the sample capillary to the LC union, ensuring that both the liner tube and sample capillary are fully butted into the LC union.

Disconnect the LC connection and feed the sample capillary through the probe, ensuring that a 0.3mm graphitised vespel ferrule (GVF/003) is fitted.

Using a Rheodyne spanner, gently tighten the adapter nut onto the probe.

Replace the probe tip and adjust so that 0.5mm of sample capillary protrudes from the probe tip.

Replace the probe end cover and tighten the grub screw to clamp the LC union.

The APcI Probe

Indications that maintenance to the APcI probe is required include:

- The probe tip assembly becomes contaminated, for example by involatile samples if the probe temperature is too low during operation (300°C).
- The appearance of chromatogram peak broadening or tailing.

  Samples that give rise to a good chromatogram peak shape in APcI (for example reserpine and common pesticides) should display peak half widths of the order 0.1 minutes for 10µl loop injections at a flow rate of 1 ml/min. The appearance of significant peak broadening or tailing with these compounds is most likely to be due to a broken fused silica capillary or probe tip heater assembly.
- Low LC pump back pressure.

  For 50:50 acetonitrile:water at a flow rate of 1 ml/min, a LC pump back pressure less than 14 bar (200 psi) is indicative of a broken fused silica capillary or a leaking connector.
- High LC pump back pressure.

  For 50:50 acetonitrile:water at a flow rate of 1 ml/min, a LC pump back pressure above 35 bar (500 psi) is indicative of a blockage or partial blockage in the fused silica capillary, in a LC connector or in the filter. It is advisable to change the inner filter pad (see "Replacing the Fused Silica Capillary" in the following pages) on a regular basis.
- Gas flow problems.

  Check all gas connections for leaks using soap solution, or a suitable leak searching agent such as Snoop.

Cleaning the Probe Tip

Remove any visible deposits on the inner wall of the probe heater with a micro-interdental brush (supplied in the spares kit) soaked in methanol:water.

Before starting an analysis:

With the probe out of the instrument, connect the nebulising gas supply line.

Select **Gas** and turn on **Nitrogen**.

Allow the gas to flow for several seconds to clear any debris from the heater.

Turn off **Nitrogen**.

Insert the probe into the source.

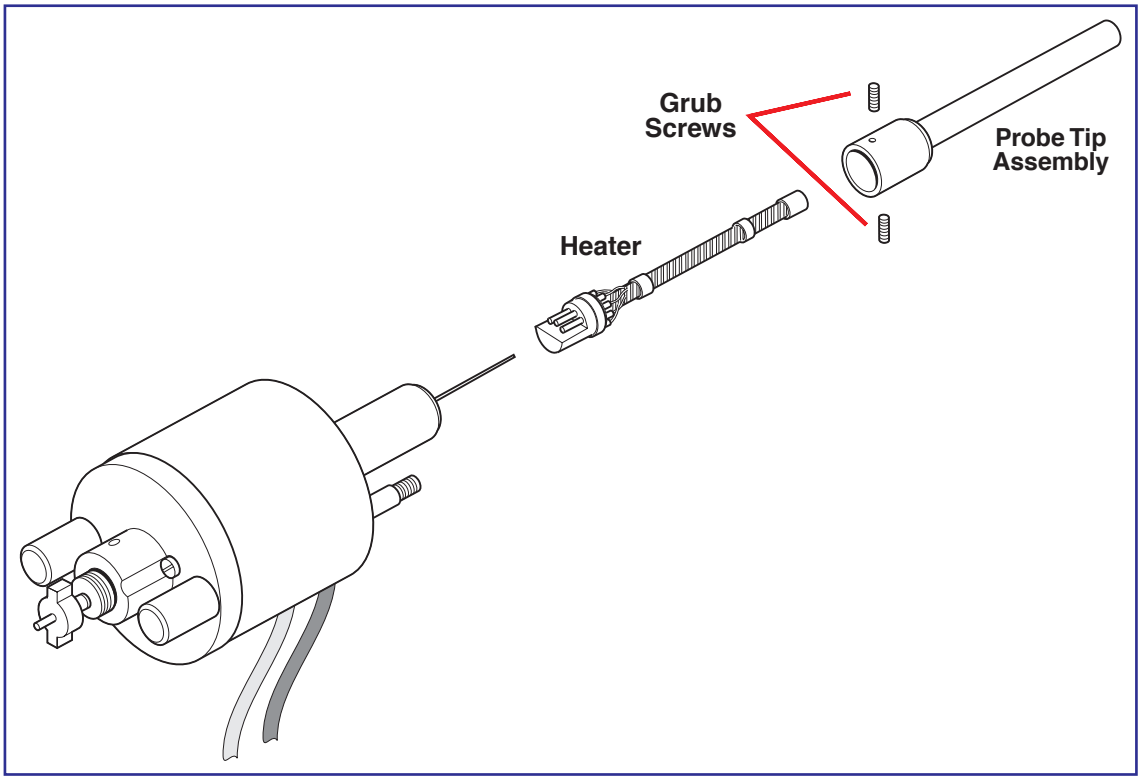
Select **Gas** and turn on **Nitrogen**.

Raise **APcI Heater** gradually, starting at 100°C and increasing in 50°C intervals to 650°C over a period of 10 minutes.

Caution: Do not set **APcI Heater** to 650°C immediately as this may damage the probe heater.

This procedure should remove any chemical contamination from the probe tip.

Replacing the Probe Tip Heater



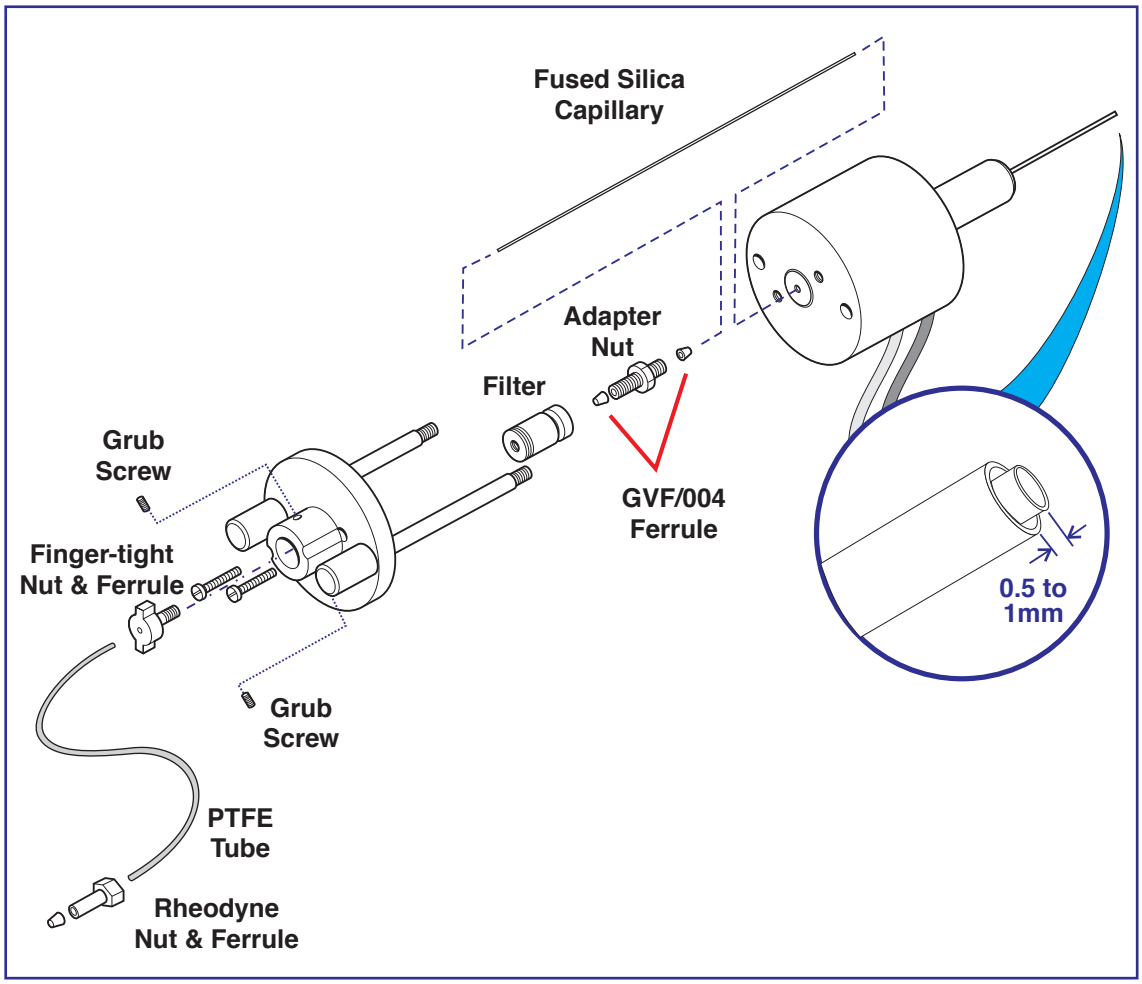
Remove the probe tip assembly by carefully loosening the two grub screws.

Disconnect the heater from the probe body by pulling parallel to the axis of the probe.

Fit a new heater assembly.

Reconnect the probe tip assembly.

Replacing the Fused Silica Capillary



With the probe removed from the source proceed as follows:

Remove the probe tip assembly and the heater, as described in the preceding section.

Remove the probe end cover by removing the two screws and the grub screw that retains the LC filter.

Loosen the filter from the adapter nut.

Unscrew the adapter nut from the probe.

Remove and discard the fused silica capillary.

Using a ceramic capillary cutter, cut a new length of 300μm o.d. × 100μm i.d. fused silica capillary, about 1 centimetre excess in length.

Using a GVF/004 ferrule and the adapter nut, connect the sample capillary to the filter ensuring that the liner tube is fully butted into the filter.

Feed the sample capillary through the probe, ensuring that a 0.4mm graphitised vespel ferrule (GVF/004) is fitted.

Using a ceramic capillary cutter, cut the capillary at the nebuliser so that between 0.5 and 1.0mm of capillary is protruding from the nebuliser.

It is important to cut the capillary square. This should be examined using a suitable magnifying glass.

Undo the adapter nut from the probe and withdraw the capillary from the probe.

Remove 20mm of polyamide coating from the end of the capillary using a flame and clean with a tissue saturated with methanol.

Carefully re-feed the sample capillary through the probe ensuring that the graphitised vespel ferrule is still fitted.

Using a Rheodyne spanner, gently tighten the adapter nut to the probe.

Replace the probe end cover and retaining screws.

Using a 1.5mm Allen key, tighten the grub screw in the probe end cover to clamp the filter.

Replace the heater and probe tip assembly.

The Analyser

Quattro LC is fitted with a pre-filter assembly that is designed to protect the main analyser by absorbing contamination from the ion beam. As a consequence the analyser quadrupoles should never, under normal working conditions, require cleaning.

The hexapole transfer lens also serves to effectively remove contamination, and the pre-filter assembly should only require cleaning on an infrequent basis. Although training will be given during installation, it is strongly recommended that this task is carried out by a Micromass service engineer or by other suitably qualified personnel.

The quadrupole assemblies of Quattro LC are finely machined and aligned assemblies which ***under no circumstances*** should be dismantled.

The Detector

The Quattro LC detector system has been designed for trouble-free operation over many years. The photomultiplier is encapsulated in its own vacuum envelope and is therefore safe from contamination and pressure surges. The conversion dynode and phosphor are also long lasting. No routine maintenance is required.

It is strongly recommended that assistance is sought from Micromass if maintenance to the detector system is thought necessary due to spikes or unacceptably high noise levels.

Electronics

Warning: There are high voltages present throughout the mass spectrometer. Extreme caution should be taken when taking measurements with a meter or an oscilloscope. In the standby mode (**OPERATE** not selected) the high voltages are switched off in the source and analyser assemblies, but high DC voltages and mains voltages remain in the power supply units.

Caution: Quattro LC's electronic systems contain complex and extremely sensitive components. Any fault finding procedures should be carried out only by Micromass engineers.

Fuses

In the following list, the designation (T) indicates a time lag fuse.

Analog PCB

Fuse No	Fuse type	Ref. No.
F1	10A (T) 20mm anti-surge TDS505	1340143
F2	10A (T) 20mm anti-surge TDS505	1340143

RF power PCB

Fuse No	Fuse type	Ref. No.
F1	5A (T) 20mm anti-surge	1340142

Power Backplane #2

Fuse No	Fuse type	Ref. No.
F7	2A (T) 20mm anti-surge TDS506	1340161
F8	2A (T) 20mm anti-surge TDS506	1340161

Pumping Logic PCB

Fuse No	Fuse type	Ref. No.
F1	2A (T) 20mm semi-delay	1340137

Power Sequence PCB

Fuse No	Fuse type	Ref. No.
F1	4A (T) 20mm anti-surge ceramic	1340164
F2	2A (T) 20mm anti-surge TDS506	1340161
F3	6.3A (T) 20mm anti-surge TDS506	1340163

Rear Panel

Fuse No	Fuse type	Ref. No.
F1	10A (T) HBC ceramic anti-surge	1340147
F2	10A (T) HBC ceramic anti-surge	1340147

Fault Finding Check List

Warning: There are high voltages present throughout the mass spectrometer. Extreme caution should be taken when taking measurements with a meter or an oscilloscope. In the standby mode (**OPERATE** not selected) the high voltages are switched off in the source and analyser assemblies, but high DC voltages and mains voltages remain in the power supply units.

Any investigation in the RF generator must be made only by a Micromass engineer.

No Beam

Refer to the relevant chapters of this manual and check the following:

- Normal tuning parameters are set and, where appropriate, readback values are acceptable.
- All necessary cables have been correctly attached to the source and probe.
- **OPERATE** is on (check the LED on the front panel).
- The source has been assembled correctly and is clean.
- The source isolation valve is open.
- There are no error messages reported by the electronics (see the viewing window at the rear of the instrument).

Unsteady or Low Intensity Beam

Should the preceding checks fail to reveal the cause of the problem check that:

- Gas and liquid flows are normal.
- The analyser pressure is less than $1x10^{-4}$ mbar.

Ripple

Peaks appear to vary cyclically in intensity when there is ripple superimposed on the peak. Possible causes are:

- Unstable power supplies in the source supplies or the RF/DC generator.
- Unstable photomultiplier supply.
- Vibration from the rotary pumps or even other equipment in the same building.

  The frequency of the ripple, measured using an oscilloscope, can often help locate the source. Mains frequency ripple, for example, points towards an unstable power supply or vibration from mains powered machinery.

High Back Pressure

For electrospray, a higher than normal back pressure readout on the HPLC pump, together with a slowing of the actual solvent flow at the probe tip, can imply that there is a blockage in the capillary transfer line or injection loop due to particulate matter from the sample. To clear the blockage:

Remove the probe from the source and increase the solvent flow to 50 µl/min to remove the blockage.

Often, injections of neat formic acid help to redissolve any solute which has precipitated out of solution.

If the blockage cannot be cleared in this fashion:

Remove the finger-tight nut and tubing from the back of the probe.

If the back pressure remains high, replace the tubing with new tube (or first try removing both ends of the tube).

If the back pressure falls, replace the stainless steel sample tube inside the probe (or try reversing the tube to blow out any blockage).

Reconnect the tubing to the probe.

The solvent flow can be readjusted and the probe replaced into the source.

To check the flow rate from the solvent delivery system, fill a syringe barrel or a graduated glass capillary with the liquid emerging from the probe tip, and time a known volume, say 10µl.

Once the rate has been measured and set, a note should be made of the back pressure readout on the pump, as fluctuation of this reading can indicate problems with the solvent flow.

For APcI a higher than normal back pressure readout on the HPLC pump can imply that, after a long period of use, the filter pad requires replacement.

General Loss of Performance

Should the preceding checks fail to reveal the source of the problem proceed as follows:

Check that the source and probe voltage readbacks vary with tune page settings.

If any of these voltages are absent check that the source and hexapole transfer lens assembly have been correctly reassembled.

Further investigation, which will require the services of a qualified service engineer, should be left to Micromass personnel.

Cleaning Materials

It is important when cleaning internal components to maintain the quality of the surface finish. Deep scratches or pits can cause loss of performance. Where no specific cleaning procedure is given, fine abrasives should be used to remove dirt from metal components. Recommended abrasives are:

- 600 and 1200 grade emery paper.
- Lapping paper (produced by 3M).

After cleaning with abrasives it is necessary to wash all metal components in suitable solvents to remove all traces of grease and oil. The recommended procedure is to sonicate the components in a clean beaker of solvent and subsequently to blot them dry with lint-free tissue. Recommended solvents are:

- Isopropyl Alcohol (IPA)
- Methanol
- Acetone

Following re-assembly, components should be blown with oil-free nitrogen to remove dust particles.

Warning: Many of the procedures described in this chapter involve the removal of possibly toxic contaminating deposits using flammable or caustic agents. Personnel performing these operations should be aware of the inherent risks, and should take the necessary precautions.

Preventive Maintenance Check List

✖ Avoid venting the instrument when the rotary pump is gas ballasting.

✖ Do not gas ballast the rotary pump for more than 2 hours under any circumstances.

✖ Under no circumstances should gas ballasting be performed during instrument operation.

For full details of the following procedures, consult the relevant sections of this chapter and/or refer to the manufacturer's literature.

Daily

- Gas ballast the rotary pump lightly for 20 minutes at the end of a day's electrospray operation.
- Gas ballast the rotary pump for 30 minutes at the end of a day's megaflow or APcI operation.

  It is normal for the rotary pump noise level to increase during gas ballasting.

Weekly

- Gas ballast for at least 30 minutes by rotating the gas ballast knob anticlockwise by 5 to 6 turns.

  When gas ballast is complete, check the rotary pump oil level and colour.

  Oil that has become noticeably red in colour should be replaced.
- Check the water chiller level and temperature (if fitted).

Monthly

- Check all cooling fans and filters.
- Change the odour element in the oil mist filter.

Three-Monthly

- Change the sorbent in the foreline trap.

Four-Monthly

- Change the mist element in the oil mist filter.
- Change the oil in the rotary pump.

  Gas ballast lightly for 30 to 60 minutes both before and after changing oil.

Reference Information

Overview

The reference files listed in this chapter have all ion intensities set to 100%. Actual ion intensities are not, of course, all 100%, but the calibration software does not take account of the ion intensities and this is a convenient way to store the reference files in the required format.

Most samples can be purchased from the Sigma chemical company. To order, contact Sigma via the internet, or by toll-free (or collect) telephone or fax:

Internet:

http://www.sigma.sial.com

This site contains a list of worldwide Sigma offices, many with local toll-free numbers.

Toll-free telephone:

USA & Canada	800-325-3010
Outside USA & Canada	++1 314-771-5750 (call collect)

Toll-free fax:

USA & Canada	800-325-5052
Outside USA & Canada	++1 314-771-5750 (call collect and ask for the fax machine)
Outside USA & Canada	++1 314-771-5757 (this is a toll call) (direct fax line)

Positive Ion

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
UBQ	Bovine Ubiquitin [U6253]	8564.85	650-1500	General
HBA	Human α globin [H753]	15126.36	700-1500	Hb analysis
SOD	Superoxide dismutase [S2515]	15591.35	900-1500	Hb (internal cal.)
HBB	Human β globin [H7379]	15867.22	800-1500	Hb analysis
MYO	Horse heart myoglobin [M1882]	16951.48	700-1600	General
PEGH1000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000		80-1000	ES+ and APcI+ calibration
PEGH2000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000 +1450		80-2000	ES+ calibration
NAICS	Sodium Iodide / Caesium Iodide mixture		20-4000	General, ES+ calibration
NAIRB	Sodium iodide / Rubidium Iodide mixture		20-4000	ES+ calibration

Horse Heart Myoglobin

Reference File: myo.ref
Molecular Weight: 16951.48

Charge State	Calculated m/z Value	Charge State	Calculated m/z Value	Charge State	Calculated m/z Value
28^+	606.419	21^+	808.222	13^+	1304.969
	616.177	20^+	848.583	12^+	1413.633
27^+	628.841	19^+	893.192	11^+	1542.053
26^+	652.989	18^+	942.758	10^+	1696.158
25^+	679.068	17^+	998.155	9^+	1884.508
24^+	707.320	16^+	1060.477	8^+	2119.945
23^+	738.030	15^+	1131.108	7^+	2422.651
22^+	771.531	14^+	1211.829		

Polyethylene Glycol

PEG + $NH4^+$

Reference Files: PEGH1000, PEGH2000

Calculated m/z Value				
63.04	459.28	855.52	1251.75	1647.99
107.07	503.31	899.54	1295.78	1692.01
151.10	547.33	943.57	1339.80	1736.04
195.12	591.36	987.60	1383.83	1780.07
239.15	635.39	1031.62	1427.86	1824.09
283.18	679.41	1075.65	1471.88	1868.12
327.20	723.44	1119.67	1515.91	1912.15
371.23	767.46	1163.70	1559.94	1956.17
415.25	811.49	1207.73	1603.96	2000.20

Sodium Iodide and Caesium Iodide Mixture

Reference File: NAICS

Calculated m/z Value				
22.9898	772.4610	1671.8264	2571.1918	3470.5572
132.9054	922.3552	1821.7206	2721.0861	3620.4515
172.8840	1072.2494	1971.6149	2870.9803	3770.3457
322.7782	1222.1437	2121.5091	3020.8745	3920.2400
472.6725	1372.0379	2271.4033	3170.7688	
622.5667	1521.9321	2421.2976	3320.6630	

Sodium Iodide and Rubidium Iodide Mixture

Reference File: NAIRB

Calculated m/z Value				
22.9898	772.4610	1671.8264	2571.1918	3470.5572
84.9118	922.3552	1821.7206	2721.0861	3620.4515
172.8840	1072.2494	1971.6149	2870.9803	3770.3457
322.7782	1222.1437	2121.5091	3020.8745	3920.2400
472.6725	1372.0379	2271.4033	3170.7688	
622.5667	1521.9321	2421.2976	3320.6630	

Negative Ion

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
MYONEG	Horse heart myoglobin [M1882]	16951.48	700-2400	General
SUGNEG	Sugar mixture of: maltose [M5885] raffinose [R0250] maltotetraose [M8253] corn syrup [M3639]		100-1500	Low mass range
NAINEG	Sodium Iodide / Caesium Iodide (or Rubidium Iodide) mixture		200-3900	ES- calibration

Horse Heart Myoglobin

Reference File: myoneg.ref

Calculated m/z Value				
891.175		1209.812		1882.490
940.741		1302.952		2117.927
996.138		1411.615		2420.632
1058.460		1540.036		
1129.091		1694.140		

Mixture of Sugars

Reference File: sugneg.ref

Calculated m/z Value				
179.06		665.21		1151.37
341.11		827.27		1313.42
503.16		989.32		1475.48

Sodium Iodide and Caesium Iodide (or Rubidium Iodide) Mixture

Reference File: naineg.ref

Calculated m/z Value				
126.9045	1026.2699	1925.6353	2825.0008	3724.3662
276.7987	1176.1641	2075.5296	2974.8950	3874.2604
426.6929	1326.0584	2225.4238	3124.7892	
576.5872	1475.9526	2375.3180	3274.6835	
726.4814	1625.8469	2525.2123	3424.5777	
876.3757	1775.7411	2675.1065	3574.4719	

Preparation of Calibration Solutions

PEG + Ammonium Acetate for Positive Ion Electrospray and APcI

Prepare a solution of polyethylene glycols at the following concentrations:

PEG 200	25 ng/µl
PEG 400	50 ng/µl
PEG 600	75 ng/µl
PEG 1000	250 ng/µl

Use 50% acetonitrile and 50% water containing 2 mmol ammonium nitrate.

Use reference file PEGH1000.

PEG + Ammonium Acetate for Positive Ion Electrospray (Extended Mass Range)

Prepare a solution of polyethylene glycols at the following concentrations:

PEG 200	25 ng/µl
PEG 400	50 ng/µl
PEG 600	75 ng/µl
PEG 1000	250 ng/µl
PEG 1450	250 ng/µl

Use 50% acetonitrile and 50% water containing 2 mmol ammonium nitrate.

Use reference file PEGH2000

Sodium Iodide Solution for Positive Ion Electrospray

Method 1

Prepare a solution of sodium iodide at a concentration of 2 µg/µl (micrograms per microlitre) in 50:50 propan-2-ol (IPA):water with no additional acid or buffer.

Add caesium iodide to a concentration of 0.05 µg/µl.

The purpose of the caesium iodide is to obtain a peak at m/z 133 (Cs^+) to fill the gap in the calibration file between m/z 23 (Na^+) and the first cluster at m/z 173, which would lead to poor mass calibration in this mass range.

Do not add more CsI than suggested as this may result in a more complex spectrum due to the formation of NaCsI clusters.

Use reference file NAICS.REF.

Method 2

Prepare a solution of sodium iodide at a concentration of 2 µg/µl (micrograms per microlitre) in 50:50 propan-2-ol (IPA):water with no additional acid or buffer.

Add rubidium iodide to a concentration of 0.05 µg/µl.

The purpose of the rubidium iodide is to obtain a peak at m/z 85 ($^{85}Rb^+$) with an intensity of about 10% of the base peak at m/z 173. Rubidium iodide has the advantage that no rubidium clusters are formed which may complicate the spectrum. Note that rubidium has two isotopes (^{85}Rb and ^{87}Rb) in the ratio 2.59:1, giving peaks at m/z 85 and 87.

Use reference file NAIRB.REF.

Sodium Iodide Solution for Negative Ion Electrospray

Either of the above solutions is suitable for calibration in negative ion mode. In both cases the first negative reference peak appears at m/z 127 (I^-) and the remaining peaks are due to NaI clusters.

Use reference file NAINEG.REF.

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